ortically installed implants with different ns. An experimental studyin rabbits

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Key words: animal study, bicortical stabilization, implant dentistry, osseointegration, surfaceconfiguration

Abstract

Objective: To study the sequential healing at bicortically installed implants with surfacemodifications by the use of fluoroboric acid and/or H_2O_2.

Material and methods: Twenty-eight albino New Zealand rabbits were used. Two recipient siteswere prepared in the tibiae bilaterally, one in the metaphysis and the second in the diaphysis regions. Four implants with different surface characteristics were randomly installed with bicorticalstabilization: (i) sandblasted and acid-etched; (ii) same surface as i, but with a substitution of the hydrofluoric acid with fluoroboric acid; (iii) same surface as i, additionally treated with H_2O_2; and (iv) same surface modified as ii, additionally treated with H_2O_2. The animals were killed after 5, 8,15, and 30 days. Ground sections were prepared for histological analyses.

Results: No statistically significant differences in osseointegration were found among various surfaces at any of the healing periods. A higher degree of osseointegration was observed at the implants placed in the metaphysis compared to those placed in the diaphysis, especially during early healing. A higher degree of osseointegration was found at sites with proximity to compact(cortical) bone when compared to the middle portion of the implants, especially in the diaphysisregion.

Conclusions: Surfaces modified with different acids or H_2O_2 resulted in similar osseointegration compared to a standard sandblasted and acid-etched surface. The portion of the bicortically installed implants in close contact with the cortical compartment presented a higher percentage ofosseointegration compared with the region in contact with the bone marrow compartment.

Osseointegration of implants installed in the alveolar bony crest occurs as a result of the communication of different cell types that express signaling molecules and proteins throughout various phases of healing (Ter- heyden et al. 2012). The process of osseointegration is composed of resorptive and appositional events, as described in an experi- mental study in dogs (Abrahamsson et al. 2004). A different pattern of healing may occur in the compact cortical and bone mar- row compartments. In fact, a faster bone apposition on implants was realized in the bone marrow spaces compared with the com- pact cortical bone compartment (Rossi et al. 2014), due to the resorptive processes that precede bone apposition in areas where the implant surface is in contact with native bone.

A number of experimental studies have shown that the surface topography plays an important role in osseointegration. Surfaces with moderate roughness reveal the highest degree of osseointegration (for review, see Wennerberg & Albrektsson 2009). The modification of a machined implant surface may be obtained in different ways, being one of the most popular the sandblasted and acid- etched method. Aiming to improve the bioactivity of the implant surface, a long series of in vitro and animal studies have been per- formed (for a review, see Wennerberg & Albrektsson 2009).

To accelerate or enhance osseointegration, various techniques have been adopted to alter the chemistry, topography, and roughness of the titanium implant surfaces. These techniques included physical, chemical, and biochemical methods (Nagassa et al. 2008; Junker et al. 2009). Among these methods, hydrogen peroxide treatment (H2O2) has been used in several studies (e.g., Nagassa et al. 2008).

Nanoengineered modifications of the sur- face or the use of bioactive additives may cre- ate an active surface that may enhance the ability to adsorb proteins and cells and increase osteoconductivity (for a review, see Variola et al. 2011 and Bressan et al. 2013).

Various acid treatments may be added to sandblasted implant surfaces such as hydro- fluoric or fluoroboric acids. The surfaces, then, may be additionally oxidized with the use of hydrogen peroxide, aiming at obtaining a layer of titanium oxide with nanotextured topography and a high concentration of hydroxyl groups (Ferraris et al. 2011).

This oxidation may enhance osseointegration, especially in the early phases of healing. Moreover, the use of fluoroboric acid instead of hydrofluoric acid as an etching agent has not been explored so far.

Hence, the aim of this study was to evaluate the sequential healing at bicortically installed implants with surface modifications using fluoroboric acid and/or H2O2.

Material and methods

The study protocol was submitted to and approved by the Ethics Committee of Murcia University, Spain (05-09-2012), which fol- lowed the guidelines established by the Council Directive of the European Union (53/2013; February 1, 2013) for animal care and experimentation and followed the ethical and legal conditions established by Royal Decree 223, March 14 and October 13, 1988, on the protection of animals used for research purposes. All surgeries were per- formed in an operating room at the Univer- sity of Murcia Research Support Service.

Implant surface features

Different processes have been applied to pro- duce modifications of a surface (ZirTi®; Swe-den & Martina, Due Carrare, Padova, Italy), which is supported by 10 years of clinical use and scientific evidence. Hence, the following four surface treatments were used:

Surface ZIRTI (ZT; Fig. 1a) – The surface was obtained by first sandblasting with zirconium microspheres at 5 at ms, dimension of grit 120 lm. Sand was constantly set with a patented fluid-vibrating process to control humidity. After that, a solution of ethylic alcohol and water demineralized for 5 min was used to clean the surface.

A subsequent treatment was carried out by immersing the implant into an initial solution of demineralized water (H_2O) and hydro-fluoric acid (HF) for 1 min. After one more cleaning process in demineralized water (H_2O), the implants were immersed in second hot solution of H_2O, sulfuric acid (H_2SO_4), and hydrochloric acid (HCl) for8 min. A final cleaning process with ethy-lic alcohol and demineralized water was applied.

Surface ZIRTI-FLUO (ZTF; Fig. 1b) – This type of process was similar to the previously described "Surface ZT," but in the first solu- tion, the hydrofluoric acid (HF) was substi- tuted with fluoroboric acid (HBF_4) for 10 min.

Surface ZIRTI-MimeTi (ZTM; Fig. 1c) –This type of process was also similar to the previously described "Surface ZT," but beforethe final cleaning process, the implants were immersed in a bath of hydrogen peroxide (H_2O_2).

Surface ZIRTI-FLUO-MimeTi (ZTFM; Fig. 1d)

– This type of process is a combination of "Surface ZTF" with the same final oxidation process mentioned for "Surface ZTM."

Clinical procedures

Twenty-eight albino New Zealand rabbits (Rabbit Farm San Bernardo, Sl, Navarra, Spain), 30–35 weeks of age and weighing 3900–4500 g, were used for the present exper-iment. The rabbits were anesthetized with an intramuscular injection of tiletamine/zolaze- pam 15 mg/kg (Zoletil 50; Virbac, Madrid,Spain) and xylazine 5 mg/kg (Rompun; Bayer,Leverkusen, Germany). Before surgery, theskin at the proximal tibia was shaved and disinfected with Betadine® (Meda Manufac- turing, Burdeos, France). Ketamine hydro- chloride (Ketolar®; Pfizer, Madrid, Spain) was administered as an anesthetic at 50 mg/kg IM. A preoperative antibiotic (Amoxicillin;Pfizer, Barcelona, Spain) was administered intramuscularly, and 3 ml of lidocaine at 2% with 0.01 mg/ml epinephrine was also
administered intramuscularly in the surgical area of each leg.

The skin of both tibiae was incised, flaps were elevated, and the proximal area was exposed, several millimeters below the ante- rior tibial tuberosity (Fig. 2a). Two experi-mental sites were identified at each tibia (Fig. 2b), one at the metaphysis and one inthe diaphysis areas and prepared for implant installation (Fig. 2c). Four implants with the same configuration (Premium; Sweden & Martina), 8.5 mm long and 3.3 mm in diame- ter, two in each tibia, each with a different modified surface, were randomly installed bi- cortically (Fig. 2d). The apex of the implants was placed in close contact with or into the cortical bone opposing the coronal corticalcompartment. Cover screws were placed on the implants, and flaps were subsequently sutured.

The animals were given 0.05 mg/kg bupr-
enorphine subcutaneously every 12 h after the operation for 3 days. Within 2–3 days, the animals resumed normal ambulation and did not show signs of pain or distress. Postop-eratively, the wounds were inspected daily for clinical signs of complications. The animals were kept in purpose-designed roomsand were fed and watered ad libitum with a standard diet. All animals were kept in indi- vidual cages at the Animal Room Service Unit, University of Murcia, Spain.

The animals were killed after 5, 8, 15, and 30 days. The same sedation and anesthesiaprotocols as for surgery were applied, and the killing was performed by means of an intra- cardiac overdose of thiopental.

Histological preparation

Individual blocks containing the implant and the surrounding hard tissues were harvested. The specimens were washed in saline solu- tion and immediately fixed in 10% buffered formalin and then processed to obtain thinground sections with the Precise 1 AutomatedSystem (Assing, Rome, Italy). The specimenswere dehydrated in an ascending series of alcohol rinses and embedded in a glycolmeth-
acrylate resin (Technovit® 7200 VLC; Kulzer,
Wehrheim, Germany). After polymerization, the specimens were sectioned, along their lon-gitudinal axis, with a high-precision diamond disk at about 150 lm, and one histological sample was ground down to about 30 lm witha specially designed grinding machine. The slides were stained with acid fuchsin and toluidine blue.

Histological examination

In an Eclipse Ci microscope (Nikon Corpora- tion, Tokyo, Japan), connected to a digitalvideo camera (Digital Sight DS-2Mv; Nikon) and a computer, the following landmarks were identified: (B) the most coronal contactof the bone to the implant and (A) the base of the implant. The implant was divided into three sections of similar length, termed basedon their position in respect of the long axis:
(i) coronal, (ii) middle, and (iii) apical portion.The percentages of (m) bone marrow, (n) newbone, (o) old bone, and (p) clot and bone deb- ris/particles in contact with the implant sur- face were measured on the entire implant length as well as on each of the three sec- tions (coronal, middle, and apical). The mea- surements were performed between B and A, using the software NIS-Elements D v.4.10 (Laboratory Imaging; Nikon Corporation) at a
magnification of 9200. The total amount of
mineralized bone was calculated as the sumof new and old bone.

The apical portion of the implant that extruded beyond the compact cortical layer was excluded from the analyses.

Randomization and data analysis

The animals were randomly assigned to oneof the four groups before surgery. The positionof the implants in the tibiae was randomly assigned using a similar scheme for all groupsso that different periods could be analyzedamong implants placed in the same position. Two very expert and well-calibrated surgeonsperformed the surgeries and were not aware ofthe type of surface used. The period of healingof each group was randomly assigned at the completion of the surgeries by a different per-son from the surgeons. The histologist was very expert and well calibrated and not aware of surface characteristics. One sample per each implant was stained and analyzed.

The primary outcome variables were new

(osseointegration) and old bone percentage.The differences between various surfaces were analyzed with the Friedman test and the Wilcoxon rank-sum test for paired obser- vations. Differences for each surface between the healing periods were analyzed with Mann–Whitney *U*-test for independent vari- ables. Differences between implants placedin the diaphysis and metaphysis, respec- tively, as well as between the three sections (coronal, middle, and apical) were also per- formed using a Wilcoxon rank-sum test. The level of significance was set at a= 0.05.

Clinical and histological outcomes

No complications occurred during the heal-ing period, and no artifacts were generated during histological processing. All implants appeared well integrated at the histologic analysis. Hence, all sites and periods of heal- ing yielded an $n = 7$ per each group analyzed.

Five-day healing

Ground sections illustrating the healing after5 days are presented in Figs 3a and 4a for themetaphysis and diaphysis zones, respectively.Woven bone was already present after 5 days of healing, forming from the parent bone of thecortical layer as well as of the trabeculae. Ahigh degree of osseointegration was found atall surfaces. Higher values of osseointegration(Figs 5a and 6a) were found at the surfaces trea-ted with hydrofluoric acid (ZT = 22.4 13.9%and ZTM = 25.6 14.8%) compared with those treated with fluoroboric acid (ZTF =17.2 7.7 and ZTFM = 13.5 7.1%). How-ever, the differences were not statistically sig-nificant. The surfaces additionally immerged into H_2O_2 provided the best results of osseoin-tegration at the surface treated with hydroflu- oric acid (ZTM = 25.6 14.8%), but also the worst results at the surface treated with fluo- roboric acid (ZTFM = 13.5 7.1%). None of the differences between surfaces was statisti- cally significant.

Old bone was present at percentages rang- ing between 17% and 20% (Figs 5b and 6a). Clot, bone debris, and very small amounts of bone particles were observed on the implant surfaces, the percentages ranging between 8 and 10% (Fig. 6a).

More osseointegration was found at the implants placed in the metaphysis compared with those placed in the diaphysis, especially in the middle and apical sections (Fig. 7). However, none of the differences were statis- tically significant. A higher degree of osseoin-tegration was seen at the apical sections of both implants placed at diaphysis and me-taphysis regions.

The total amount of mineralized bone incontact with the implant surface was

35.6 4.2% and 40.9 12.8% at the diaphy- sis and metaphysis regions, respectively. The difference, however, was not statistically sig- nificant (Fig. 8).

Eight-day healing

Ground sections illustrating the healing after8 days are presented in Figs 3b and 4b for themetaphysis and diaphysis zones, respectively.Newly formed bone percentages (Figs 5a and 6b) increased between 5 and 8 days ofhealing to an extent ranging between 11 and20% (mean, 15.7%), especially at the ZTFMsurface (+20%). All differences between thevalues of the 5- and 8-day period of healingwere statistically significant, with the excep-tion of the surface ZT, however, that showedthe highest values at 8-day period of healing.The percentage of osseointegration tented tobecome more similar, being 37.9 18.7%,33.1 15.7%, 36.9 16.2%, and 33.5 9.3% at the surfaces ZT, ZTF, ZTM, and ZTFM, respectively. None of the differences in osseo-integration among surfaces was statisticallysignificant.

Old bone percentages decreased at a rate of about 3–5% compared to the previous period of healing, the percentages ranging between 13 and 16% (Figs 5b and 6b). Small amounts of bone particles and bone debris were found ranging between 4 and 6% of the total sur- face examined at implants (Fig. 6b).

Again, a higher degree of osseointegration was found at the implants placed in the me- taphysis compared with those in the diaphy- sis regions. The difference was statisticallysignificant (Fig. 7). The lowest degree of osseointegration was found in the middle section of the implant placed in the diaphysis region. The differences between the middle section of the implant placed in the diaphysis against the coronal and apical sections of the same implants were statistically significant.Moreover, the difference between the osseo- integration in the middle section of the me- taphysis and of the diaphysis wasstatistically significant.

The total amount of mineralized bone-to-implant contact was 44.3 7.0% and however, were statistically significant. The differences between surfaces tended to fur-ther decrease compared to the previous peri- ods. The percentages of osseointegration were52.0 12.6%, 50.2 9.4%, 48.4 15.8%, and 48.8 15.4% at the surfaces ZT, ZTF, ZTM, and ZTFM, respectively. None of thesedifferences between surfaces was statistically significant.

Old bone was still present in percentagesof about 9–10.5% (Figs 5b and 6c). Onlysmall amounts of free bone debris and parti-cles were found at this stage of healing (about1.7% as mean value among surfaces; Fig. 6c).A slightly higher amount of new bone was found at implants placed in the metaphysiscompared with those placed in the diaphysisregions (Fig. 7). The differences were not sta-tistically significant. The lowest degree ofosseointegration was found in the middlesection of the implant placed in the diaphysisregion. The differences between the middlesection and the coronal and apical sections ofthe same implant were statistically significant.

The total amount of mineralized bone incontact with the implant surface (Fig. 8) was also similar between implants placed in the diaphysis (57.8 12.8%) and metaphysis (61.3 11.7%) regions, respectively.

Thirty-day healing

Ground sections illustrating the healing after 30 days are presented in Figs 3d and 4d forthe metaphysis and diaphysis zones, respec- tively.

Newly formed bone percentages (Figs 5aand 6d) were lower at this time of healing at all surfaces. No differences between this per- iod and the previous healing period were, however, statistically significant. The per- centages of osseointegration were, at this time of healing, 38.1 8.6%,

43.9 20.3%, 43.0 14.9%, and 42.2 16.7% at the sur- faces ZT, ZTF, ZTM, and ZTFM, respec- tively. None of the differences between surfaces or healing periods was statistically significant.

Old bone was still present in a small per- centage, <5% (Figs 5b and 6d). No free bone debris or particles were found at this stage of healing (Fig. 6d).

New bone was found to be slightly, but 55.0 8.8% at the diaphysis and metaphysis implants, respectively. This difference did not reach statistical significance (Fig. 8).

Fifteen-day healing

Ground sections illustrating the healing after15 days are presented in Figs 3c and 4c for the metaphysis and diaphysis zones, respec- tively.

Newly formed bone percentages (Figs 5aand 6c) increased again between 8 and15 days of healing to an extent ranging between 11 and 17% (mean, 14.5%). Noneof the differences between 8 and 15 days, statistically not significantly, higher at the implants placed in the metaphysis whencompared to the diaphysis region (Fig. 7). Thelowest degree of osseointegration was found in the middle section of the implant placedin the diaphysis. The differences between the osseointegration of the middle section and found at the rough compared to the turned surfaces.

In another experiment (Rossi et al. 2014), implants with a sandblasted, acid-etched sur- face were installed in the alveolar bony ridge in Labrador dogs. The sequential phases of osseointegration were assessed after 5, 10, 20,and 30 days from implant installation. After 10 and 20 days of healing, 13.2% and 38.5% of osseointegration were identified, respec- tively. After 30 days of healing, 50.6% of osseointegration was achieved. The different pattern of healing between cortical and bone marrow regions was also evaluated. It wasshown that a higher degree of osseointegra- tion concomitant with a lower percentage of old bone was found in the bone marrow compartment compared to the cortical compartment. This confirmed the assumption that osseointegration was faster in zones wherebone apposition is not preceded by bone resorption (Abrahamsson et al. 2004).

Comparing the osseointegration on impla- nts in the dog models with that of humanbiopsy studies (Bosshardt et al. 2011; Langet al. 2011), it has to be realized that the osseointegration process took approximately double as long in humans. After 4 weeks of healing, osseointegration on SLA® surfaces of32.4% was observed. However, at 6 weeks, osseointegration had reached 62%.

In the present study, an enhanced rate of osseointegration was observed in the early phases of healing at the standard surface trea- ted with hydrogen peroxide (ZTM).

This is in agreement with other *in vitro* and *in vivo* studies (Nagassa et al. 2008; Zhang et al. 2011). In an experiment *in vitro* (Nagassa et al. 2008), titanium disks were treated with a solution of 30% of hydrogen peroxide for different periods, from 0 to 24 h or from 1 to 4 weeks. Using an atomic force microscope, a scanning electron microscope, and a profilometry device, it was shown that the lower period of treatment produced modi- fications of surface roughness at a nanomet- ric range while longer times produced modifications in the micrometric range. It was concluded that 24 h was the best treat- ment period with H_2O_2 to obtain the best condition to be beneficial to protein adsorp- tion, cells attachment, and proliferation.

These advantages were subsequently shownin a study in dogs (Zhang et al. 2011). The surface of disks and of implants with a SLA® surface was treated with alkali or hydrogen peroxide. Surface bioactivity was performed on cell attachment and proliferation, alkaline phosphatase (ALP) activity, and calcium deposition on the sample surfaces. It was that of the coronal and apical sections of the same implant were statistically significant. Also, at the implants placed in the metaphy- sis, the lowest degree of osseointegration was found in the middle section. The difference was not statistically significant when com-pared to the coronal section of the same implants.

The total amount of mineralized bone-to- implant contact (Fig. 8) was similar between implants placed in the diaphysis (43.4 9.0%) and those placed in the metaphysis (44.9 10.3%) regions.

Discussion

The present study evaluated the sequential osseointegration at bicortically installed implants with surfaces of different character- istics. It was evident that different surface treatments affected bone apposition and resorption. However, in the present study, these effects were not of a magnitude thatresulted in statistically significant differ- ences. Hence, it has to be accepted that the surface treatments performed in the implants installed in the present study had only lim- ited impact in the first period of osseointegra-tion. The reason for these presumably similar outcomes may be found in the fact that the surface modifications are, indeed, only minor in nature and hence would have a very lim- ited effect on the osseointegration process. It has to be kept in mind that either of the sur- faces tested yielded an osseointegration per- centage of approximately 50% after 15 days, indicating successful osseointegration andclinical stability. Most likely, the moderately rough surfaces tested resulted all in optimal outcomes.

The fact that there were no statistically significant differences in the early healing parameters does not mean that all the sur- faces were equal. To test equality would necessitate a different design of study with a much increased study population. Thiswould be difficult to achieve in animal stud- ies owing to animal ethical and cost reasons. For all practical reasons and for clinical appli-cation, all the four implant surfaces tested in the present study yielded satisfactory osseo- integration already after 2 weeks.

In the present study, relatively high per-centages (13.5–25.6%) of osseointegration were found already after 5 days, especially at the surface modified with H_2O_2 (25.6%). Osseointegration then increased over time until 15 days and, subsequently, was slightly reduced again (from 50 to 40%). Simulta- neously, old bone was resorbed, but was still present after 1 month of healing (<5%). This pattern of healing is in agreement with other studies performed in animals (Abrahamsson et al. 2004; Rossi et al. 2014) and humans (Bosshardt et al. 2011; Lang et al. 2011). In an experimental study in dogs (Abrahamsson et al. 2004), implant geometry was modified to obtain a trough within the thread region ofa prototype implant so that a wound chamber was formed. Two surfaces were used, one turned and one moderately rough (SLA®). In 20 Labrador dogs, 160 of these experimental implants were installed and biopsies were harvested after various periods of healing between 2 h and 12 weeks. Initially, the wound chamber was found filled with a coag-ulum that was substituted by a provisionalmatrix and by new bone. After 1 week of healing, newly formed bone was already found in close contact with the rough sur-face. The proportion of osseointegration incre-ased over time up to 65% after 4–6 weeks of healing. Subsequently, a slight decrease (to 60%) was noted at both surfaces. A higher bone-to-implant contact percentage was always concluded that both treatments significantly increased the surface wettability and cell attachment percentage, ALP activity, and the quality of calcium deposition in comparison with standard SLA® and Ti-control surfaces. Moreover, both treated surfaces obtained a good osseointegration.

In the present study, new bone formation progressed at a higher speed at the implants placed in the metaphysis compared with that in the

diaphysis. This may be related to the denser pattern of trabecular bone in the former compared to the latter regions of the tibia, a fact that may have helped to pro- mote osseointegration. On the contrary, the bone marrow included in the diaphysis scar- cely contributed to bone formation, as shown by the lower degree of osseointegra- tion on the surface in the middle section of implants placed in the diaphysis compared to that found in the coronal and apical sections.

The extreme regions of the implants werein close contact with pristine bone (bicortical stabilization), a condition that may have pro- moted osseointegration on the implant sur-face.

In conclusion, surfaces modified with different acids or H_2O_2 resulted in similarosseointegration compared to standard sand- blasted and acid-etched surfaces. The portion of the bicortically installed implants in close contact with the cortical compartment pre- sented a higher percentage of osseointegration compared with the region in contact with the bone marrow compartment.

Acknowledgements: This study wassupported by a grant from Sweden & Martina SRL, Due Carrare, Padova, Italia, by ARDEC, Ariminum Odontologica SRL, Rimini, Italia,and by the Clinical Research Foundation (CRF) for the Promotion of Oral Health, Brienz, Switzerland. It is highly appreciated the competent contributions of Dr. Sara Ferraris, Dipartimento di Scienza Applicata eTecnologia-DISAT Istituto di Ingegneria e Fisica dei Materiali – Institute of Materials Engineering and Physics, Torino, for information provided about the treatments applied to the surfaces and for the related images that have been used in the present article. The contribution of Dr. Martina Caneva in the writing process is also highly appreciated. All the authors declare no conflict of interest with the material presented.

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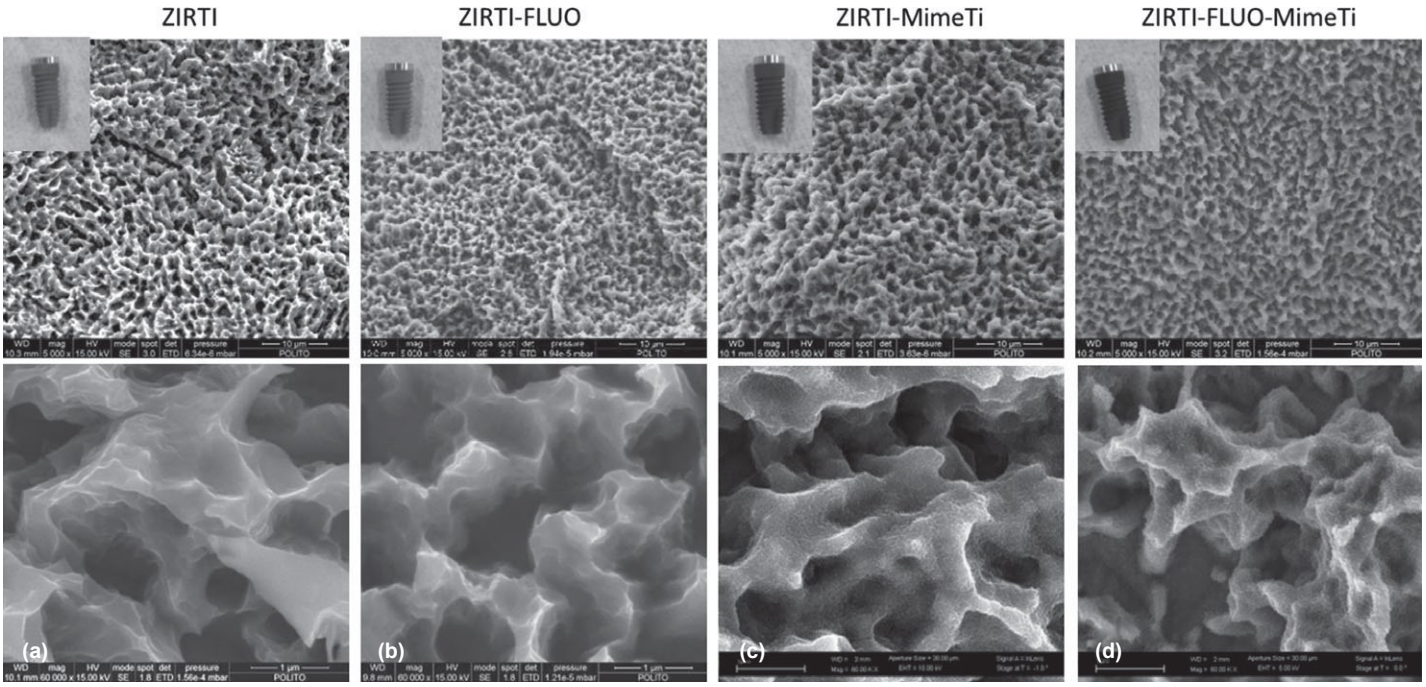

Fig. 1. SEM images of the different implanted surfaces at low (50009; images above) and high (60,0009; images below) magnification. Courtesy of Dr. Silvia Spriano and Dr. Sara Ferraris.

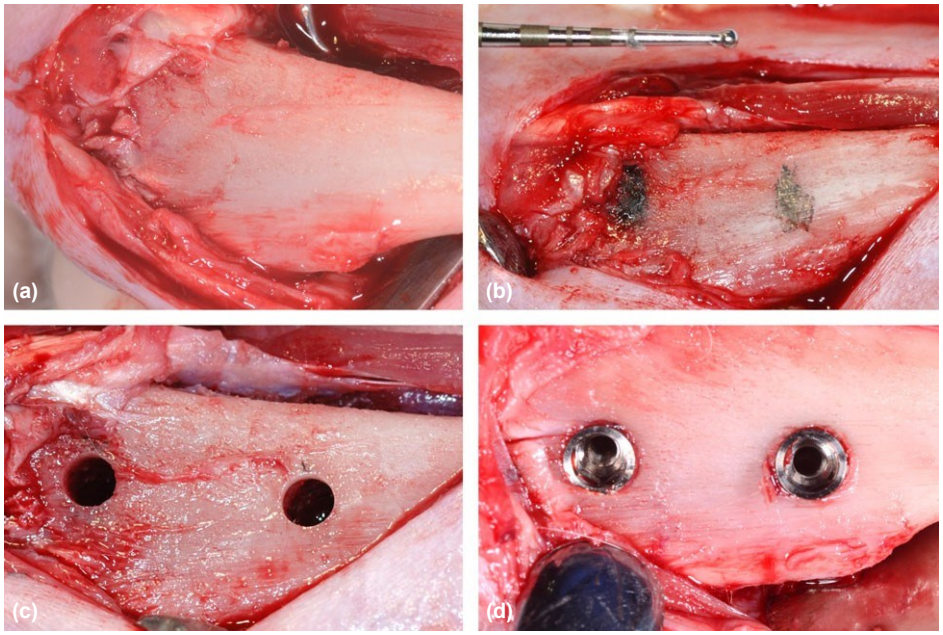

Fig. 2. Clinical view. (a) The skin at the medial section of the tibia was incised, flaps were elevated, and the proxi-mal area was exposed. (b) Two experimental sites were identified, one in metaphysis and one in the diaphysis areas. (c) The sites were prepared for implant installation. (d) Two implants each tibia were installed.

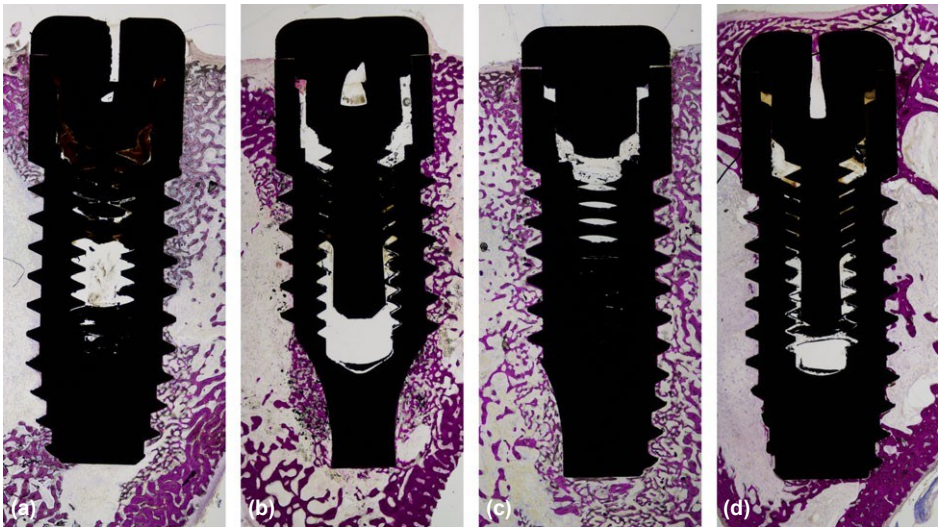

Fig. 3. Ground sections illustrating the healing of implants installed in the metaphysis areas after different periods: (a) 5 days; (b) 8 days; (c) 15 days; (d) 30 days.

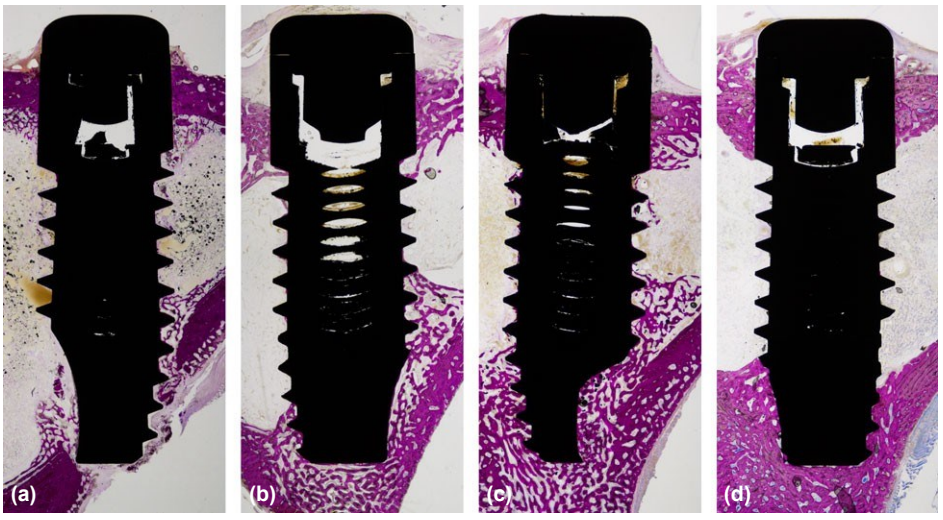

Fig. 4. Ground sections illustrating the healing of implants installed in the diaphysis areas after different periods: (a) 5 days; (b) 8 days; (c) 15 days; (d) 30 days.

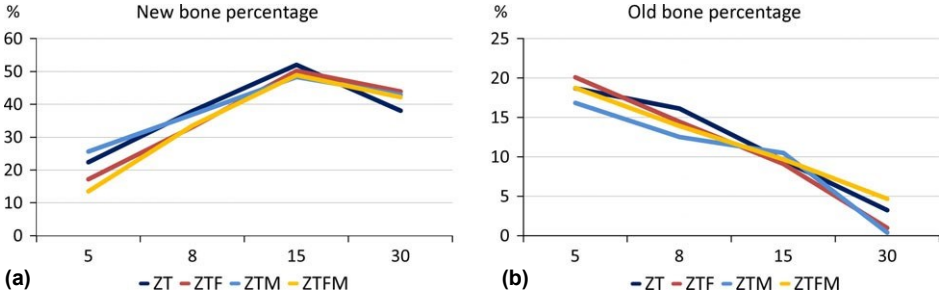

Fig. 5. Graphics reporting the amount of new bone (a) and old bone (b) at various periods of healing for the different surfaces (n = 7). ZT = ZIRTi; ZTF = ZIRTI-FLUO; ZTM = ZIRTI-MimeTi; ZTFM = ZIRTI-FLUO-MimeTi.

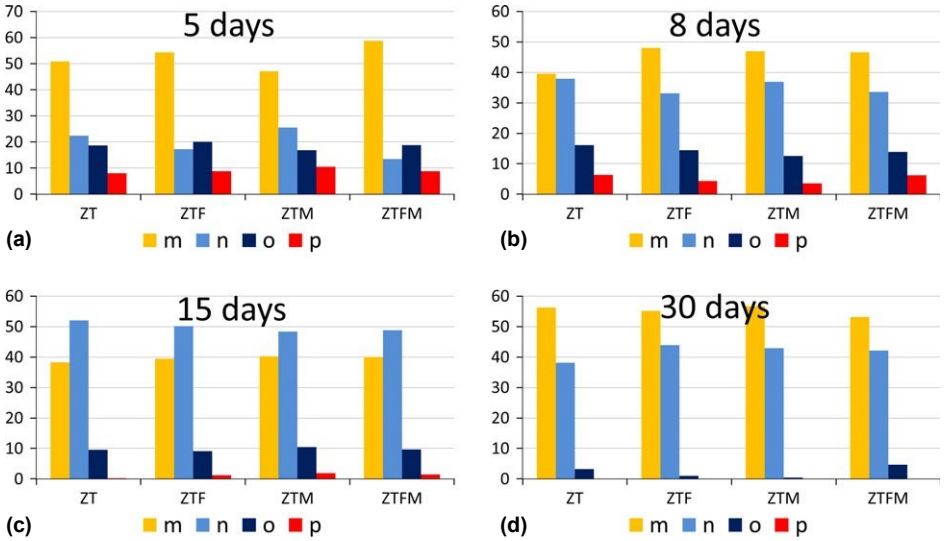

Fig. 6. Graphics reporting the amount of (m) bone marrow, (n) new bone, (o) old bone, and (p) clot and bone debris/ particles after the various periods of healing at the different surfaces (*n* = 7). ZT = ZIRTi; ZTF = ZIRTI-FLUO; ZTM = ZIRTI-MimeTi; ZTFM = ZIRTI-FLUO-MimeTi.

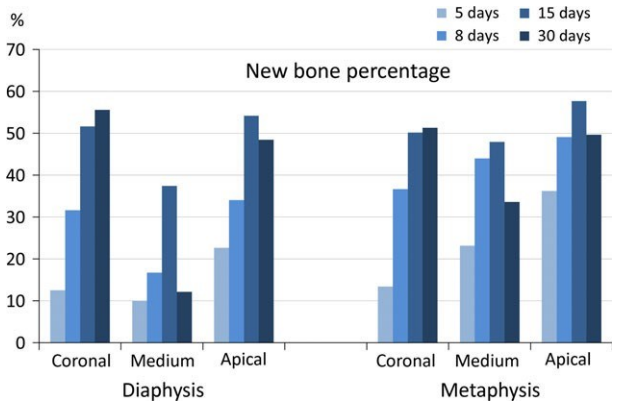

Fig. 7. Graphic illustrating new bone percentage at the various periods of healing at implants placed in diaphysisand metaphysis divided into three sections: coronal, middle, and apical. Mean values of the two surfaces placed inthe same location (diaphysis or metaphysis) were obtained per each animal at the various periods of healing (*n* = 7).

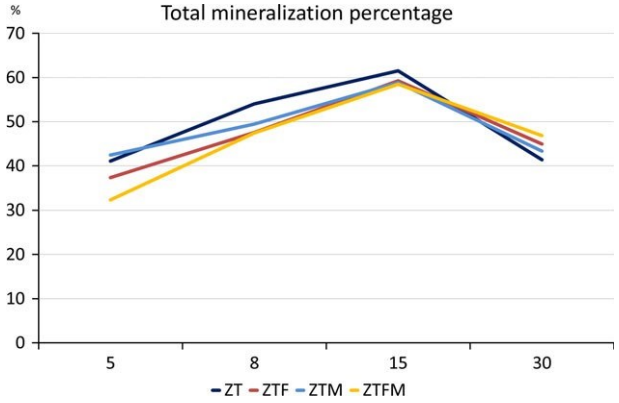

Fig. 8. Graphics reporting the total amount of mineralized bone (new + old) at the various period of healing for thedifferent surfaces (*n* = 7). ZT = ZIRTi; ZTF = ZIRTI-FLUO; ZTM = ZIRTI-MimeTi; ZTFM = ZIRTI-FLUO-MimeTi.