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Analysis of Bone Tissue Healing around Titanium Implant Surface Treated with *Tio* Sandblasted after Three and Six Weeks Used Different Histological Methods – a Study in Rabbits

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ABSTRACT

Objective: The aim of this study was to observe the in vivo cellular reaction and calcium deposition around dental implants after applying three histological techniques.
Methods: Six adult New Zealand rabbits, with an average weight of 3.5 kg, were included. Twenty-four cylindrical implants with an internal hex were used in the experiment. The implants were sandblasted with titanium micro- particles and then were conditioned with maleic acid. Calcein was injected subcutaneously at two different time points to assess calcium deposition on the bone - at the first and second weeks in examination group 1 (exam1); and at fourth and fifth weeks in examination group 2 (exam2) - with three animals in each group. Each implant was cut into 3 slices, and each slice was used for a different histological technique: one for light microscopy, one for fluorescence, and one for scanning. The samples were analysed and compared with the reactions at the interface of the bone and implant after 3 and 6 weeks.
Results: The results demonstrated different reactions in bone tissue being observed at the time corresponding to the exam1 group, mainly intense cellular proliferation and bone matrix production. The exam2 group demonstrated organisation and the maturation of the newly form tissue. The clinical significance is that, under appropriate conditions, restorative procedures may begin after 6 weeks.
Conclusion: The implants showed large corticalised areas, and they featured lamellar bone after 6 weeks. Calcium deposition occurred with greater intensity between the 3th and 6th weeks after implantation.

KEYWORDS: Osseointegration; dental implants; titanium oxide sandblasted; bone mineralization; histological methods.

INTRODUCTION

Proper shape and surface characteristics of an implant can provide great benefits in bony tissue healing, both accelerating and enhancing the tissue' capacity (Aparício et al., 2011). Several studies have shown that the level of osseointegration is correlated with the degree of surface roughness. This relationship can be attributed to factors such as physico-chemical properties, which enhance wettability and adhesion and promote cell proliferation. These factors can increase the contact area between the bone and implant, improving their biomechanical interaction (Chiesa et al., 2007; Hallgren et al., 2003; Le Guehennec et al., 2007; Simon et al., 2005).
Cellular behaviours, such as adhesion, morphological changes, proliferation and differentiation, are determined mainly by surface characteristics. These characteristics include the chemical composition, surface roughness, hydrophilicity, texture and morphology present in titanium (Anselme et al., 2000a, b; Lincks et al., 1998; Nebe et al., 2010; Zhu et al., 2004). Recently, it has been shown that changes in the physicochemical properties of titanium can result in significant modulation of cell recruitment, adhesion, inflammation and bone remodelling activities, in addition to regulation of the bone formation response (Wennerberg and Albrektsson, 2009; Omar, 2010).

The micro-irregularities present in implants can induce different healing processes (Kaiser et al., 2006). In many ways, the ability of the cells to move depends on the surface to which they are exposed. The topography of metallic surfaces determines the type of implant healing response. Furthermore, implant surfaces with pores typically increase the quality of osseointegration. These porous interfaces increase the growth within ridges, forming a mechanical interlock (Giavaresi et al., 2003). It has also been observed that cellular union, cell morphology and cell proliferation are influenced by surface treatment, with a significant increase in the activity of osteoblastic cells on surfaces with porous coatings (Simon et al., 2005).

The actual manufacture of clinically applied implants has undergone mechanical blasting coupled or not, with acid etching, bioactive coatings, anodised and, more recently, laser-modified surfaces (Cochran et al., 1998; Jansen et al., 1993; Palmquist et al., 2010). These implants have been extensively documented in vivo, including in long-term clinical studies and experimental histological and biomechanical evaluations using animal models. For more information on the clinical results with commercially available implants, the reader is referred to the cited literature (Esposito et al., 2005; Albrektsson and Wennerberg, 2004).

Also, differentiation of the cells surrounding implants should be the primary concern, as long as calcified bone around the implant is desired. However, in addition to the initial stages of the cellular phenomenon of ossification (proliferation, differentiation and cell multiplication), a factor of crucial importance for implants is bone matrix mineralisation around them. This process generally follows three stages: (1) incorporation by woven bone formation; (2) adaptation of bone mass to load (lamellar and parallel-fibered deposition); and (3) adaptation of bone structure to load (bone remodelling) (Schenk and Buser, 1998). An undisturbed healing period, along with adequate quality and quantity of bone available at the implant site, is essential for

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proper osseointegration (Wood and Vermilyea, 2004). However, mineralised-tissue deposition by osteogenic cells on titanium has not been examined thoroughly in vitro because the reactive products of von Kossa or alizarin red staining, which are normally used to visualise mineralised tissue, are difficult to recognise on the surface of titanium (Groessner-Schreiber and Tuan, 1992). Fluorescence reagents, such as tetracycline (Rolle, 1965), calcein (Rasmussen, 1975), and alizarin red (Jensh and Brent, 1966), are used to label mineralised tissue, but this labelling can only be observed by fluorescent microscopy, which is not suited to the observation of wide areas.

The determination of the optimal time to wait before loading implants depends on bone healing, which only occurs after bone matrix production and calcification. Thus, this study aimed to evaluate in vivo, using different histological methods, the bone remodelling and deposition of calcium on titanium surfaces at the 3th and 6th weeks after implantation.

MATERIALS AND METHODS

Six adult New Zealand rabbits (*Oryctolagus cuniculus*), each weighing approximately 3.5 kg, were used for this study. The animals were kept in individual cages with controlled light and were given the ad libitum diet conventionally used in vivaria. This study had the approval of the ethics committee of the Federal University of Santa Maria (Brazil), under number 133/2011.

A total of 24 cylindrical implants, 4 mm in diameter and 8 mm in length, were manufactured by Implacil De Bortoli - Dental Products (São Paulo, Brazil). The implants were surface-treated by sandblasting with microparticles of titanium oxide (TiO), with an average particle size of 180 μm; then, the implants conditioned with maleic acid, packaged and readied for commercialisation. The implants were placed on both sides of the tibia. A milling installation was performed, following the proper procedure as indicated by the manufacturer, to a depth of 8 mm, where the implants were placed manually. Four implants were placed in each animal, two in each tibia.

Before surgery, the animals were administered general anaesthesia with an intramuscular injection of ketamine (35 mg/kg; Atener Pharmaceutica, Brazil). Then, the muscle relaxant Rompum (5 mg/kg, Bayer, Brazil) and the tranquiliser Aceptan (0.75 mg / kg, Univet, Brazil) were injected intramuscularly. Additionally, 1 ml of local aesthetic (3% felypressin prilocaine, Astra, Mexico) was injected subcutaneously at the surgical site to enhance analgesia and control bleeding. Postoperatively, we used a single dose of 600,000 IU of Benzetacil. After surgery, the animals were placed in individual cages with 12- hour cycles of light, a controlled temperature (21°C) and the ad libitum diet commonly used in laboratories. Three animals were euthanised after 3 weeks, representing examination group 1 (exam1). Three additional animals were euthanised after another 6 weeks, representing examination group 2 (exam2). Euthanasia was performed with an intravenous overdose of ketamine (2 ml) and xylazine (1 ml), and then both tibiae were removed.

During the period of osseointegration, the bone marker calcein was injected subcutaneously at two different times: at the 1st and 2nd weeks after implantation in the exam1 group; and at the 4th and 5th weeks after implantation in the exam2 group. These injections of calcein were performed to analyse the deposition of calcium at different time points. After processing of the samples, the markers were identified by fluorescence microscopy.

For the histological evaluation, three different methods were used: light microscopy, fluorescence and SEM. The samples were processed first by initially setting them in a solution of formaldehyde (10%, 48 h), then by washing them in water (12 h) and finally by gradually dehydrating them by immersion in a sequence of alcohols (60%, 70%, 80%, 99%, 24-56 h). After drying, the samples were embedded in Technovit 7200 resin VLC (Kultzer & Co, Wehrhein, Germany). Then, they were sectioned with a cutter blade (Isomet 2000, Buehler, Germany), yielding three sections of each implant in the longitudinal direction, with one section from each implant being used for each type of evaluation. The slides were then ground to a thickness of 50 μm. All of the histological procedures were performed in the laboratories of Biotecnos in Santa Maria. The slides were then coated with gold, and micrographs were obtained using the back-scattered electron (BSE) detector of an XL30 Philips scanning electron microscope.

The images were analysed qualitatively by an investigator with extensive experience in bone histology. Additionally, the number of osteocytes present inside the coils of each implant was counted using Image Tool software, version 3.0 for Windows. This count was performed for each implant, and the results were then statistically compared between the groups using Student's *t*-test ($P < 0.05$).

RESULTS

After 3 and 6 weeks, all of the implants had osseointegrated, and microscopic analyses of the implant surfaces verified good new bone formation. In sections analysed by light microscopy, we observed a fair amount of bone tissue growing on the surface of the implants after 4 weeks. However, the bone tissue was still organising itself. There were large numbers of fibres and cells present in those areas (Fig. 1).

SEM images showed that a large part of the implant was in contact with the forming bone, along with some corticalisation (Fig. 2).

In the samples evaluated by fluorescence microscopy, the deposition of calcium was visible by the presence of a small amount of green colour. This characterisation revealed a small amount of calcium deposition 3 weeks after implantation (Fig. 3).

After 6 weeks, the samples exhibited a structural conformation that was more complete and uniform. The formation of a lamellar bone structure could be observed in all of the samples. Analysis of the cuts revealed osteons at the implemented interface, indicating the organisation of collagen fibres and nutritional support. Moreover, we observed several areas with cellular activity in the bone

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tissue near the surfaces of the implants, along with lamellar organisation with many canaliculi (Fig. 4).

The SEM analysis of the 6-week samples revealed a large amount of corticalised bone tissue in contact with the implants (Fig. 5). Furthermore, we observed with this histological technique that there were adequate contact and corticalised bone growth at the bone-implant interface, which was probably stimulated by the surface characteristics.

For fluorescence microscopy in the exam2 group, calcein (green) showed the deposition of calcium ions that corresponded to growth and bone formation, which appeared to be more intense than in samples from the exam1 group (Fig. 6). These results indicated that more extensive calcification of new bone occurred in large part after 3 weeks post-implantation. The colour appeared more intense around the centre of the lamellae, where the presence of blood vessels provides nutrition.

The number of osteocytes present in each implant was an average of 73,50 with an SD of 5,30 for the samples at 3 weeks and 69,60 with an SD of 4,72 for the samples at 6 weeks. There was no significant difference between the groups (P = 0,087), only better distribution (organisation) of cells in the studied areas.

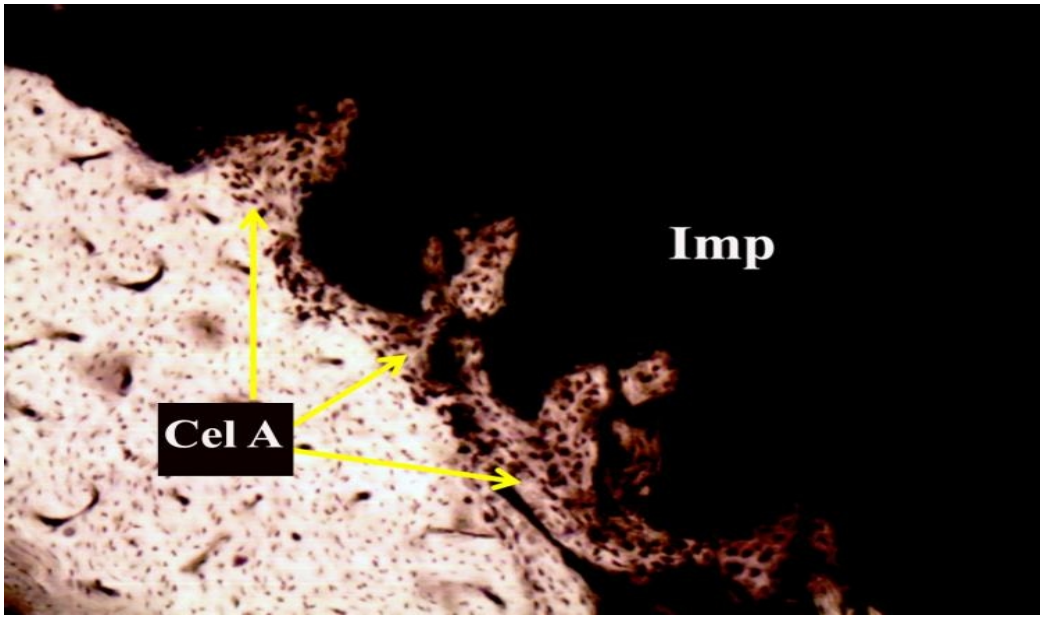

Figure 1: Photomicrograph of implant (Imp) exam1 group after 3 weeks (stained with HE; x40). The presence of intense cellular activity (Cel A) is indicated by yellow arrows.

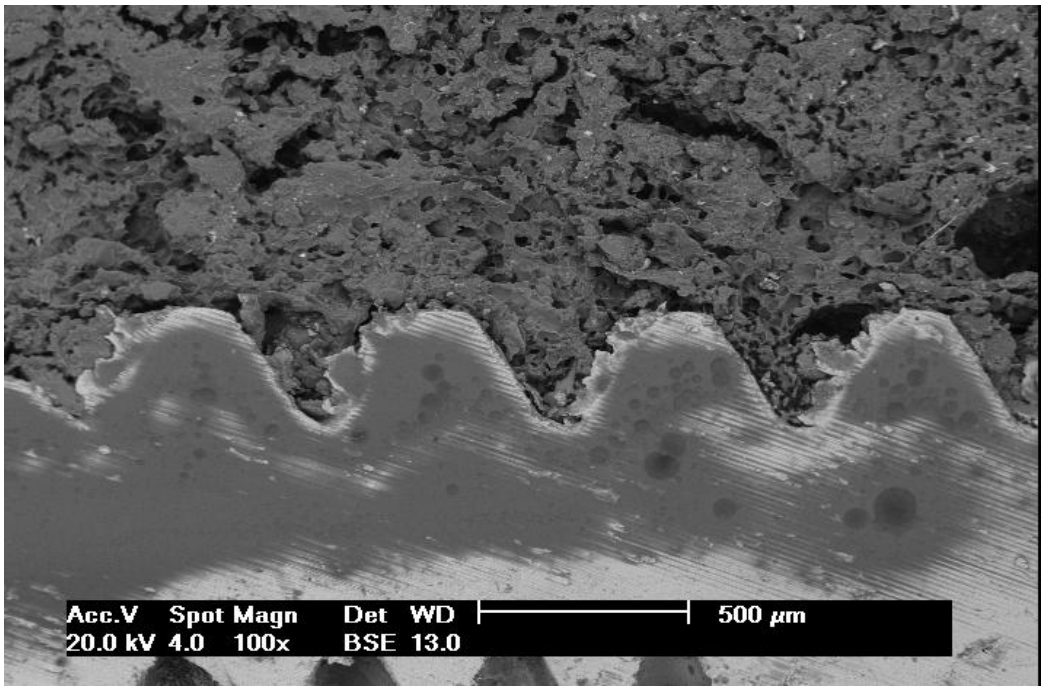

Figure 2: SEM image of an implant exam1 group with an area of trabecular

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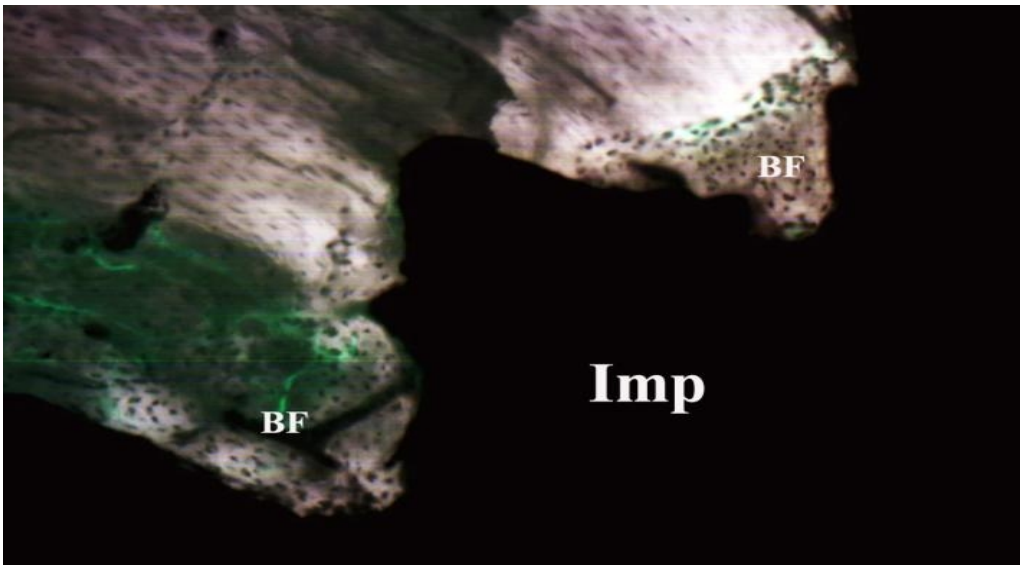

Figure 3: Fluorescence photomicrographs obtained from implant exam1 group, showing a small deposit of calcium in the bone around the implant after application of calcein (stained green; x100).

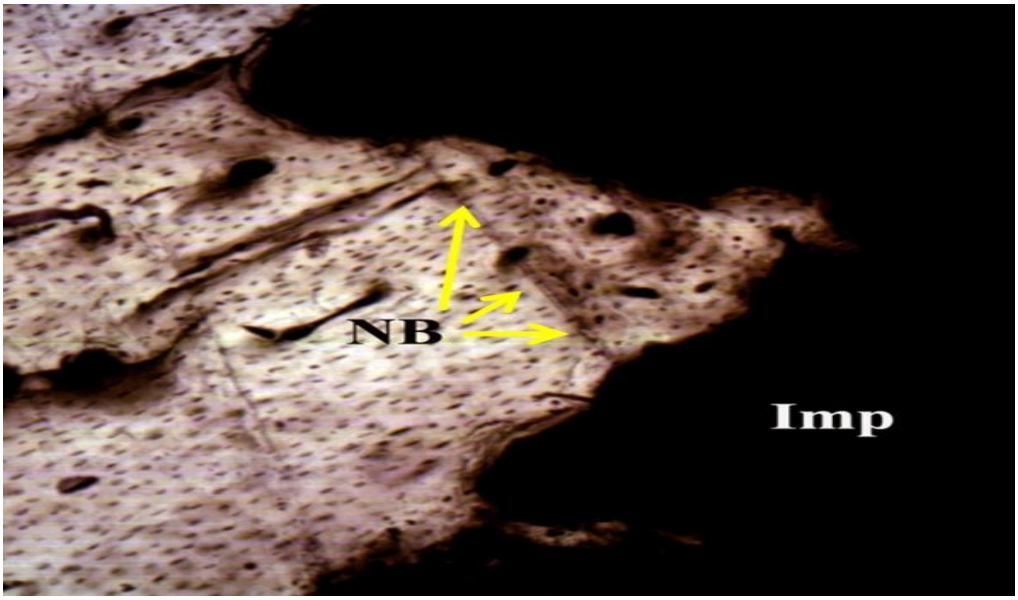

Figure 4: Photomicrograph of implant (Imp) exam2 group after 6 weeks (stained HE; x100). We can observe the presence of major cellular activity (NB) and good organisation of the fibres, forming lamellae around the implants.

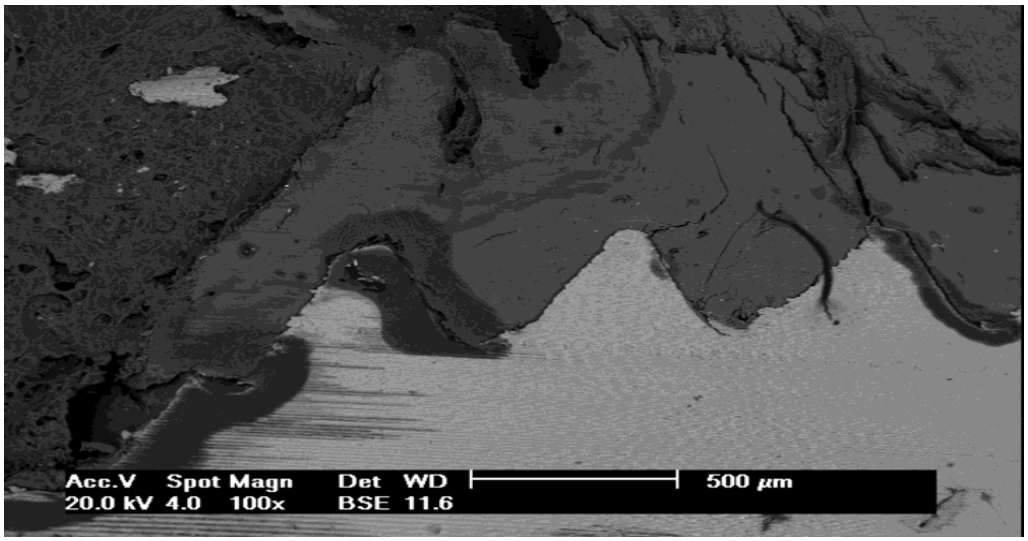

Figure 5: Analysis of the 6th weeks sample by SEM imaging, showing suitable bone growth over the surface of the implant, with a large amount of corticalised bone (x100).

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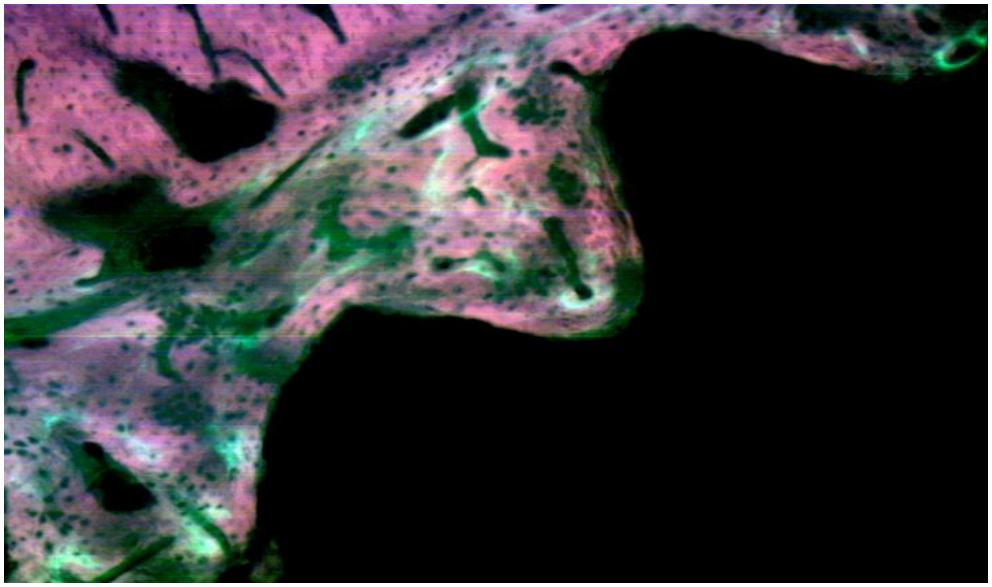

Figure 6: Fluorescence photomicrograph obtained from implant exam2 group, showing intense deposition of calcium in the bone around the implant after the application of calcein (stained green). This experiment determined the period of deposition to be between the 4th and 6th weeks. The highest intensity of the marker can be observed around the blood vessels (x40).

DISCUSSION

In recent years, there have been major changes in the concepts and in our knowledge of the processes involved in the biology and physiology of tissues related to implantation. The evolution of drawings, the different types of materials used for the surfaces of biocompatible implants and new surgical techniques, which are considered responsible for the success rates and predictability of implant prostheses, have all helped to change the concepts of tissue biology and physiology in the context of implementation. However, there is an ongoing search for the optimisation of these techniques, with shorter procedures and greater success rates. This study analysed, using different histologic methods, the quality and quantity of osseointegration on surfaces that were blasted with microparticles of titanium oxide and conditioned with acid. The methods used to characterise the outcomes included light microscopy, fluorescence and SEM. With light microscopy, the quality of contact of the new bone and the cellular proliferation in the implanted area were analysed. With these images, the cellular organisation in forming new bone around the samples could be seen at two different time points, and lamellar conformation and adequate structural features appeared after 6 weeks.

Observations of the structural orientation of the implants have been described in some studies (Kaiser et al., 2006; Piattelli et al., 1998). One other important observation was the large number of osteons found in bone tissue after 6 weeks in the implanted area, which was also observed in other studies (Allegrini et al., 2006). The use of bone markers is very important for identifying the deposition of calcium during the growth of new bone tissue (Giavaresi et al., 2003). With fluorescence markers, the amount of bone deposition that occurs over time can be observed, and other information can be obtained from samples (Martin et al., 1996). In this study, only calcein was used as a marker for the identification of bone growth, which was indicated by the green colour on the photomicrographs. The excitation wavelength of ?uorescent calcein reagents is relatively long, at 497 nm (Goto et al., 2003).

Furthermore, the decision to use only one marker was undertaken to facilitate its identification on the images, which would have been more difficult had multiple markers been used in the same group of animals. The intense presence of the fluorescence marker in all of the samples of the exam2 group indicated that there was an adequate response induced after stimulation by the placement of the implant in the bone and that there was adequate availability of minerals, especially calcium, in the bloodstream. This finding confirmed good maturation of the tissue at different times. However, tissue growth, organisation and bone calcification occurred with greater intensity between the 3th and 6th weeks. It was possible to observe and demonstrate, using different histological techniques, that the most intense cellular reaction occurred during the first 3 weeks, with little calcium deposition during that period. In the following weeks, there was a reduction in the numbers of cells in these areas, as well as increased calcium deposition.

These findings are in agreement with other studies, which showed that 4 months after implantation, the periimplant fibrous tissue almost disappeared, while new bone had began functional remodelling. Six months after implantation, newly formed bone was so mature and functionally remodelled that the boundaries of the original bony defect were barely visible by microscopy (Ciocan et al., 2011). A number of different types of studies have found that the phenomenon of osseointegration occurs with higher quality and better security when the implants have better primary stability (Donath and Breuner, 1982; Thomas and Cook, 1985). In the present study, all of the implants had good primary stability, which was probably one of the determining factors of the large amount of corticalised bone tissue in contact with the implant surfaces, indicating

adequate stimulus for the organisation of events related to osseointegration. The complex role of implant surface topography and its impact on the healing response affect the biological criteria that could guide the design and development of future tissue-implant surface interfaces (Stanford, 2010). Thus, within the limitations of this study, it was possible to observe that the events related to the bone healing process around of titanium surfaces occur in an organised manner over time. In situations where the implants are installed in healthy bodies, appropriate conditions and material presenting appropriate characteristics (eg. surface), restorative procedures may begin after 6 weeks.

CONCLUSION

Different histologicaltechniques can be used to determine the osseointegration of implants, and the unification of the information presented provides greater understanding of the studied process. Thus, it was possible to conclude that practically all cell proliferation occurs during the first 3 weeks, and in the subsequent weeks, these cells organised in only one circular direction, forming the lamellae and resulting in the mineralisation of new tissue after 6 weeks.

REFERENCES

1. Aparicio C, Rodriguez D, Gil FJ. Variation of roughness and adhesion strength of deposited apatite layers on titanium dental implants. Mater Sci Eng. 2011;31:320-324.
2. Chiesa R, Giavaresi G, Fini M, Sandrini E, Giordano C, Bianchi A, et al. In vitro and in vivo performance of a novel surface treatment to enhance osseointegration of endosseous implants. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103:745-756.
3. Hallgren C, Reimers H, Chakarov D, Gold J, Wennerberg A. An in vivo study of bone response to implants topographically modified by laser micromachining. Biomaterials 2003;24:701-10.
4. Le Guehennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater. 2007;23:844- 854.
5. Simon M, Lagneau C, Moreno J, Lissac M, Dalard F, Grosgogeat B. Corrosion resistance and biocompatibility of a new porous surface for titanium implants. Eur J Oral Sci. 2005;113:537-545.
6. Anselme K, Bigerelle M, Noel B, Dufresne E, Judas D, Iost A, et al. Qualitative and quantitative study of human osteoblast adhesion on materials with various surface roughnesses. J Biomed Mater Res. 2000a;49:155-166.
7. Anselme K, Linez P, Bigerelle M, LeMaguer D, Le Maguer A, Hardouin P, et al. The relative influence of the topography and chemistry of TiAl6V4 surfaces on osteoblastic cell behavior. Biomaterials 2000b;21:1567-77.
8. Lincks J, Boyan BD, Blanchard CR, Lohmann CH, Liu Y, Cochran DL, et al.. Response of MG63 osteoblast-like cells to titanium and titanium alloy is dependent on surface roughness and composition. Biomaterials. 1998;19: 2219-2232.
9. Nebe JB, Jesswein H, Weidmann A, Finke B, Lange R, Beck U, et al. Osteoblast's sensitivity to topographical and chemical features of titanium. Mater Sci Forum. 2010;638-642 and 652-657.
10. Zhu X, Chen J, Scheideler L, Reich R, Geis-Gerstorfer J. Effects of topography and composition of titanium surface oxides on osteoblast responses. Biomaterials. 2004;25, 4087-4103.
11. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res. 2009;20:172-184.
12. Omar O, Lenneras M, Svensson S, Suska F, Emanuelsson L, Hall J, Nannmark U, Thomsen P. Integrin and chemokine receptor gene expression in implant-adherent cells during early osseointegration. J Mater Sci Mater Med. 2010;21:969-980.
13. Kaiser JP, Reinmann A, Bruinink A. The effect of topographic characteristics on cell migration velocity. Biomaterials. 2006;27:5230-5241.
14. Giavaresi G, Fini M, Cigada A, Chiesa R, Rondelli G, Rimondini L, et al. Mechanical and histomorphometric evaluations of titanium implants with different surface treatments inserted in sheep cortical bone. Biomaterials. 2003;24:1583-1594.
15. Cochran DL, Schenk RK, Lussi A, Higginbottom FL, Buser D. Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: A histomorphometric study in the canine mandible. J Biom Mat Res. 1998;40:1-11.
16. Jansen JA, Wolke JGC, Swann S, van der Waerden JPCM, de Groot K. Application of magnetron-sputtering for producing ceramic coatings on implant materials. Clin Oral Implants Res. 1993;4:28-34.
17. Palmquist A, Omar O M, EspositoM, Lausmaa J, Thomsen P. Biomechanical, histological, and ultrastructural analyses of laser micro- and nano-structured titanium alloy implants: A study in rabbit. J Biomed Mater Res. A. 2010;92:1476-1486.
18. Esposito M, Coulthard P, Thomsen P, Worthington HV. The role of implant surface modifications, shape and material on the success of osseointegrated dental implants. A Cochrane systematic review. Eur J Prosthodont Restor. Dent. 2005;13:15-31.
19. Albrektsson T, Wennerberg A. Oral implant surfaces: Part 2--review focusing on clinical knowledge of different surfaces. Int J Prosthodont. 2004;17:544-564.
20. Schenk RK, Buser D. "Osseointegration: a reality", Periodontology 2000. 1998;17:22-35.
21. Wood MR, Vermilyea SG, and Committee on research in fixed prosthodontics of the Academy of Fixed Prosthodontics. A review of selected dental literature on evidence-based treatment planning for dental implants: report of the Committee on research in ?xed prosthodontics of the Academy of Fixed Prosthodontics. J Prost Dent. 2004;92: 447-462.
22. Groessner-Schreiber B, Tuan RS. Enhanced extracellular matrix production and mineralization by osteoblasts cultured on titanium surface in vitro. J Cell Sci. 1992;101:209-17.

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23. Rolle GK. Histochemistry of bone formation in normal and tetracycline- treated chick embryos I. lipids. Arch Oral Biol. 1965;10:393-405.

24. Rasmussen P. Effect of oxytetracycline and puri?ed calcein (DCAF) on the apposition and mineralization of rat incisor dentin. Scand J Dent Res. 1975;83:233-7.

25. Jensh RP, Brent RL. Rapid schedules for KOH clearing and alizarin red S staining of fetal rat bone. Stain Technol. 1966;41:179-183.

26. Piattelli A, Degidi M, Paolantonio M, Mangano C, Scarano A. Residual aluminum oxide on the surface of titanium implants has no effect on osseointegration. Biomaterials. 2003;24:4081-4089.

27. Piattelli M, Scarano A, Paolantonio M, Iezzi G, Petrone G, Piattelli A. Bone response to machined and resorbable blast material titanium implants: an experimental study in rabbits. J Oral Implantology. 2002;28:2-8.

28. Allegrini Jr. S, Rumpel E, Kauschke E, Fanghanel J, Konig Jr B. Hydroxyapatite grafting promotes new bone formation and osseointegration of smooth titanium implants. Ann Anat. 2006;188:143-151.

29. Martin JY, Dean DD, Cochran DL, Simpson J, Boyan BD, Schwartz Z. Proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63) cultured on previously used titanium surfaces. Clin Oral Implants Res. 1996;7:27-37.

30. Goto T, Kajiwara H, Yoshinari M, Fukuhara E, Kobayashi S, Tanaka T. In vitro assay of mineralized-tissue formation on titanium using ?uorescent staining with calcein blue. Biomaterials. 2003;24:3885-3892.

31. Ciocan LT, Miculescu F, Miculescu M, Patrascu I. Dental Implant Surface Enhancement and Osseointegration. In: Turkyilmaz, I. (Ed.) Implant Dentistry - A Rapidly Evolving Practice. InTech, Rijeka, 2011: pp. 505-530.

32. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. J Oral Pathol. 1982:11:318-326.

33. Stanford CM. Surface modification of biomedical and dental implants and the processes of inflammation, wound healing and bone formation. Int J Mol Sciences. 2010:11:354-369.

34. Thomas KA, Cook S. An evaluation of variables influencing implant fixation by direct bone apposition. J Biomed Mater Res. 1985;19:875-901.

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