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Early detachment of titanium particles from various different surfaces of endosseous dental implants

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Abstract

Titanium (Ti) endosseous dental screws with different surfaces (smooth titanium—STi, titanium plasma-sprayed—TPS, alumina oxide sandblasted and acid-etched—Al-SLA, zirconium oxide sandblasted and acid etched—Zr-SLA) were implanted in femura and tibiae of sheep to investigate the biological evolution of the peri-implant tissues and detachment of Ti debris from the implant surfaces in early healing. Implants were not loaded. Sections of the screws and the peri-implant tissues obtained by sawing and grinding were analysed by light microscopy immediately after implantation (time 0) and after 14 days. All samples showed new bone trabeculae and vascularised medullary spaces in those areas where gaps between the implants and host bone were visible. In contrast, no osteogenesis was induced in the areas where the implants were initially positioned in close contact with the host bone. Chips of the pre-existing bone inducing new peri-implant neo-osteogenesis were surrounded by new bone trabeculae. The threads of some screws appeared to be deformed where the host bone showed fractures. Ti granules of 3–60 μm were detectable only in the peri-implant tissues of TPS implants both immediately after surgery and after 14 days, thus suggesting that this phenomenon may be related to the friction of the TPS coating during surgical insertion.

Keywords: Titanium implants; Titanium detachment; Osseointegration; Histology; Ultrastructure

1. Introduction

Histologically, titanium (Ti) has been demonstrated to be a highly biocompatible material [1,2] on account of its good resistance to corrosion, absence of toxic effects on macrophages and fibroblasts, and lack of inflammatory response in peri-implant tissues [1,3–5].

On the other hand, clinical studies have reported hypersensitivity and allergic reactions to Ti [6,7], increase in Ti serum concentration [8], as well as the presence of the metal in the urine [9], peri-implant tissues or other organs [9–11]. Small Ti particles of unloaded implants have also been found inside the peri-implant medullary spaces of some animals 3 months after surgery; these metal debris have been hypothesised to increase Ti dispersion in blood vessels, on account of the high vascularisation of medullary tissues [12].

The surface of any material implanted in the living body can change over time [13]. It has been pointed out that Ti ions may be released from the implant surface, following corrosion, wear, and mechanically assisted electrochemical processes such as fretting corrosion, stress corrosion and corrosion fatigue [2,10,14].

Immersion tests have demonstrated that Ti is clearly eluted from some organic acids [15], and exposure to organic acids is unavoidable in the living body [16]. The release of Ti in some organs has also been seen in the absence of wear [10].

If on the one hand corrosion of Ti surfaces in the living body appears to be related to the type of corrosion in terms of hydrogen evolution and oxygen diffusion [16], on the other hand, the dispersion ratio of small Ti particles in peri-implant tissues is not clearly known. The data reported above suggest that the increasing clinical use of Ti and Ti-alloy implants in dentistry and

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orthopaedics makes Ti release a matter of topical interest. In recent years, the Ti surface of endosseous dental implants has been continuously modified to improve osseointegration. Porous coatings have been developed to enhance bone ingrowth [17]. The shear strength of implants with a modified sandblasted surface (SLA implants) has been shown to be about five times higher than that of implants with a smooth surface [18]. Histological studies have confirmed that SLA implants have a higher percentage of bone-to-implant contact than plasma-sprayed (TPS) implants [19].

When investigating new Ti implant surfaces, clinicians should take into account that any modification of the Ti implants surface may lead to corrosion and dispersion of Ti particles in peri-implant tissues. In a previous study [12] by backscattered electron imaging and EDX analysis we detected Ti particles in peri-implant tissues surrounding unloaded TPS implant surface.

The present authors investigated the early histological events occurring in a sheep model at the interface between different surfaces of unloaded Ti implants and the pre-existing bone tissue. The aim of this study was to assess peri-implant bone responses to the different Ti implant surfaces and evaluate the dispersion of Ti particles, if any, in peri-implant tissues during the early phases of the healing process.

2. Materials and methods

Twenty-eight endosseous Ti implant conic screws (3.8 mm in outer diameter and 8 mm in length) (Or-Vit Castelmaggiore-Bologna, Italy) with different surfaces [smooth titanium—STi, titanium plasma-sprayed—TPS, alumina oxide (particles diameter: 100 μm) sandblasted and acid-etched—Al-SLA, zirconium oxide (particles diameter: 120 μm) sandblasted and acid-etched—Zr-SLA] were implanted in the femoral and tibial diaphyses of two mongrel sheep aged 3–4 years. Twelve implants (three for each group) were inserted in one sheep and 16 (four for each group) in the remainder.

The animals were anaesthetised according to a standardised protocol: premedication with intramuscular injection of 10 mg/kg b.w. ketamine (Ketavet 100, Farmaceutici Gellini, SpA, Aprilia, Italy), 0.3 mg/kg b.w. xylazine (Rompun, Bayer AG, Leverkusen, Germany) and subcutaneous injection of 0.0125 mg/kg b.w. atropine sulphate; induction with intravenous injection of 6 mg/kg sodium thiopentone (2.5% solution, Pentothal, Hoechst AG, Germany); maintenance with O_2, N_2O and 1–2.5% halothane under assisted ventilation (Servo Ventilator 900 D, Siemens, Germany). A 3.5 mm-diameter tungsten drill was used to pre-drill the holes in each diaphysis. No screw tap was used. The screws were tightened to the final insertion torque of 1.7 ± 0.1 Nm and then implanted on the right- and left-hand side of each animal.

Antibiotics (cefalosporin, 1 g/day for 5 days) and analgesics (ketoprofen 500 mg/day for 3 days) were administered postoperatively.

The sheep with 12 implants and the sheep with 16 implants were euthanised with intravenous administration of Tanax (Hoechst, Frankfurt am Main, Germany) under general anaesthesia immediately after implantation (time 0) and therefore 14 days. All the implants with the surrounding peri-implant tissues were isolated, removed and fixed in 10% buffered formalin solution (pH 7.2) for histological and ultrastructural analyses. Some samples were then dehydrated in ethanol and embedded in methyl methacrylate. Sections measuring 60–100 μm in thickness were obtained by sawing and grinding operations (Saw and Grinding, Remet, Bologna, Italy); they were then stained with toluidine blue and acid fuchsin and finally observed with a light microscope.

Some unstained methylmethacrylate-embedded sections were also mounted on stubs with carbon bioadhesive film, gold/palladium-coated and observed with a Philips 515 Scanning Electron Microscope (SEM: Philips 515, Eindhoven, Holland) fitted with secondary electron (SE) and back-scattered electron (BSE) probes at voltages of 10–12 kV.

All the procedures involving the sheep were performed strictly following Italian and European Law on animal experimentation (Law by Decree, 27 January, 1992 no. 116 in accordance with the EEC rules and Animal Welfare Assurance No. #A5424-01 of the National Institute of Health (NIH-Rockville, Maryland, USA).

3. Results

No surgical complication was encountered during implant insertion in both sheep and the animal sacrificed 14 days after surgery survived the whole post-surgical period without developing any infection.

3.1. Time 0

Some surface areas of the removed implants were seen to be in close contact with the pre-existing bone. The threads of some screws appeared to be deformed where the host bone showed some fractures lines. On the other hand, other areas at the bone–implant interface showed gaps of 190–270 μm where blood cells and chips of host bone were visible. None of the implants revealed any detachment of material from the implant surface, with the sole exception of the TPS implants. Some small Ti granules detached from the implant surface could be

observed at the bone–implant interface in proximity to the surface of the TPS implants (Figs. 1 and 2).

3.2. Fourteen days post-implantation

A new calcified tissue was visible around some areas of all the endosseous Ti implants. In particular, some newly formed trabecular bone including large osteocytes and well-vascularised medullary spaces developed where gaps of 180–260 μm were observed between the implant surface and pre-existing bone. These new bone trabeculae were covered with aligned, cuboidal osteoblasts producing osteoid tissue and developed from the host bone surface, even though they were also detectable on the implant surfaces (Fig. 3). A line of flattened cells with interposition of cuboidal cells aligned along the implant surface could be observed where connective

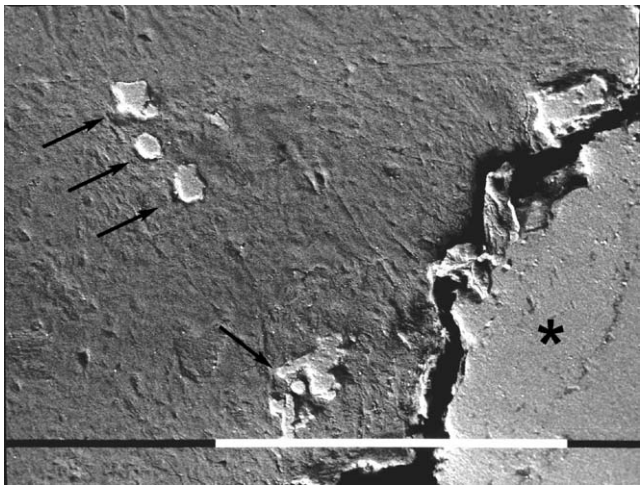

Fig. 2. TPS implant removed immediately after implantation. Back-scattered SEM observation. Ti granules (arrows) detached from the implant surface (*) are visible in the peri-implant gap. Bar = 100 μm.

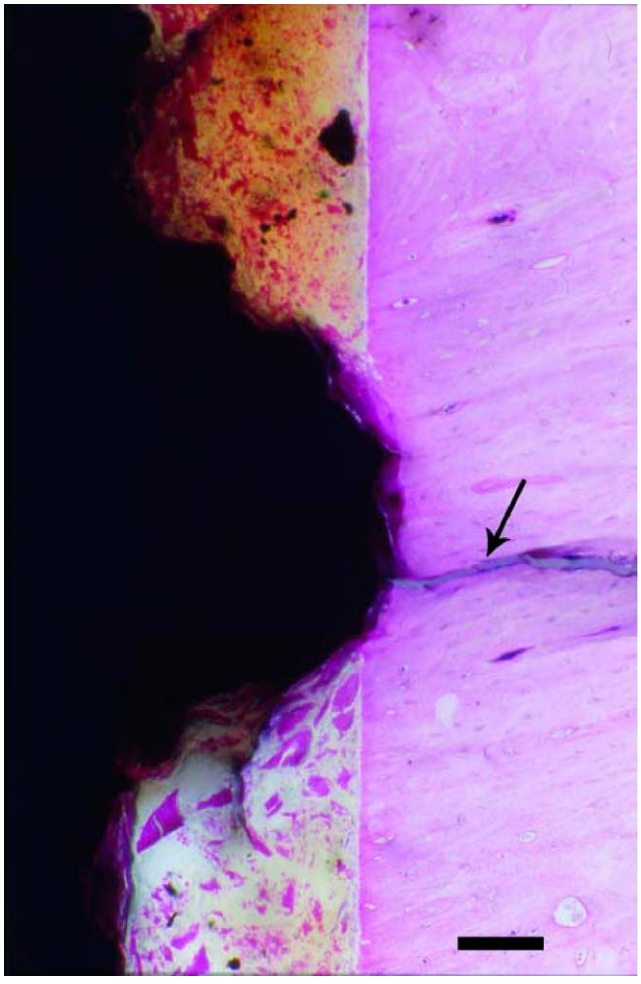

Fig. 1. TPS implant removed immediately after implantation. Light microscopy. The fracture line is visible where the thread is in close contact with the host bone (arrow). In the peri-implant space many Ti granules and blood plasma are observable (upper side). Some host bone chips are detectable in the gap between the implant and host bone (lower side). Bar = 100 μm.

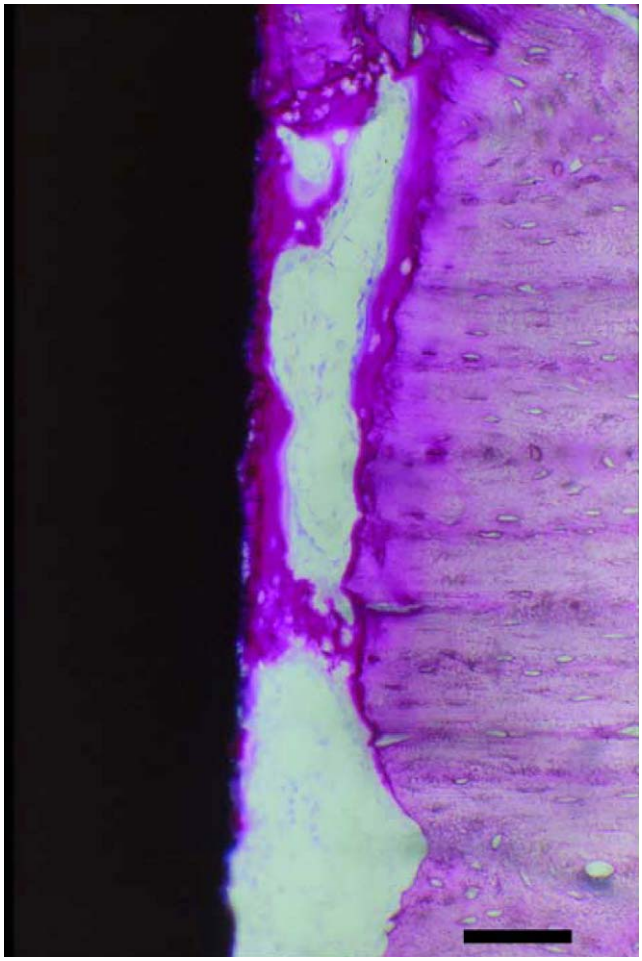

Fig. 3. Al-SLA implant 14 days after implantation. Light microscopy. New bone trabeculae in direct contact with the implant surface and highly vascularised medullary spaces are detectable where gaps of 180–260 μm are seen between the implant surface and host tissue. Cuboidal osteoblasts lining on the osteoid tissue can be observed. Bar = 100 μm.

tissue was present in the gaps between the implant and host bone (Fig. 4). A few multinucleated giant cells were also visible. There was good evidence of trabecular osteogenesis especially near the implant portions protruding inside the medullary canal of the femoral and tibial diaphyses. In these areas, between the implant and pre-existing bone, some isolated bone chips, derived from the host bone and presumably produced by the bur action during surgery, were completely included in the new trabecular bone or partially covered (Fig. 5). In contrast, no new calcified or connective tissue was observed in areas of close contact between the implants and host bone (Fig. 6). Some threads of the screws in close contact with the pre-existing bone also appeared to be deformed and bent. At the tip of the threads some fracture lines were visible in the host bone. Bone fragments were also detectable around the implant surface (Fig. 7).

No granules or small particles of Ti detached from the implant surface were seen in the peri-implant tissue, except for the TPS implants, where some Ti granules with a diameter of 9–45 μm appeared in the peri-implant tissue and were included in the newly formed trabecular bone or found in proximity to the blood vessels of the medullary peri-implant connective tissue (Figs. 8–10).

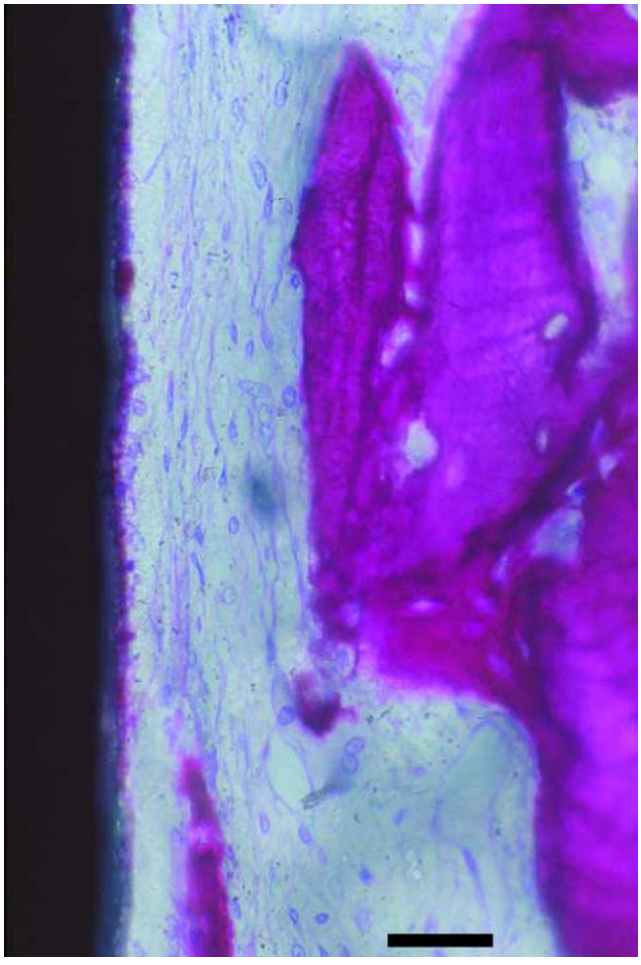

Fig. 4. Zr-SLA implant 14 days after implantation. Light microscopy. New calcified bone trabeculae are observable next to the implant and flattened cells with interposition of cuboidal cells aligned along the implant surface. Bar = 50 μm.

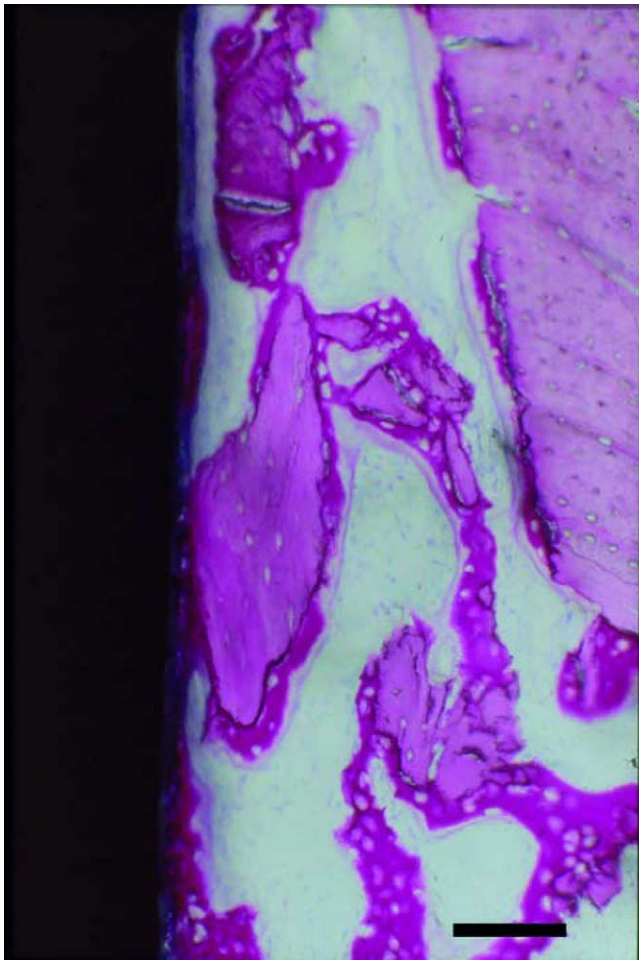

Fig. 5. Al-SLA implant 14 days after implantation. Light microscopy. New bone traculae are in tight contact with the implant surface and around some isolated host bone chips. Bar = 100 μm.

4. Discussion

Extensive and close contact between the bone and the implant with no fibrous tissue interposition has been defined as ''osseointegration''. This condition is the primary requirement for the long-term success of dental implants [20,21].

Most clinical failures occur in the early post-surgical stages [22,23] and an excessive interfacial micromotion during the healing stage has been shown to be potentially detrimental for the osseointegration process [24–26]. Moreover, cortical bone has been demonstrated to be an important anatomical site for the initial fixation and support of the occlusal forces [27–29]. The primary

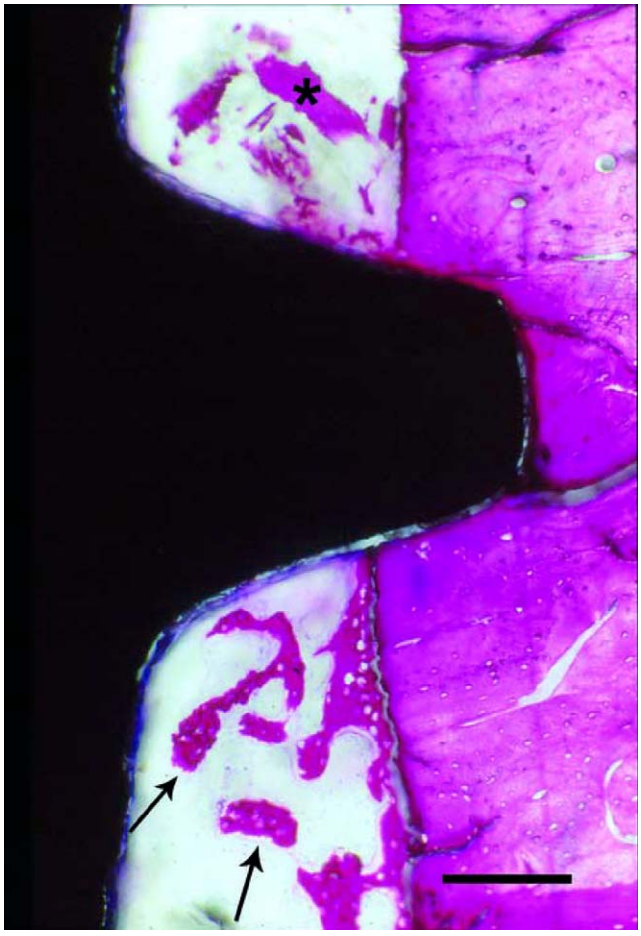

Fig. 6. STi implant 14 days after implantation. Light microscopy. Where the thread of the implant is in close contact with the pre-existing bone no new calcified or connective tissue is visible. New bone trabeculae can be seen around the implant where a gap is observed between the implant and host bone (arrows). Isolated host bone chips (*) presumably produced by the bur action during surgery. Bar = 200 μm.

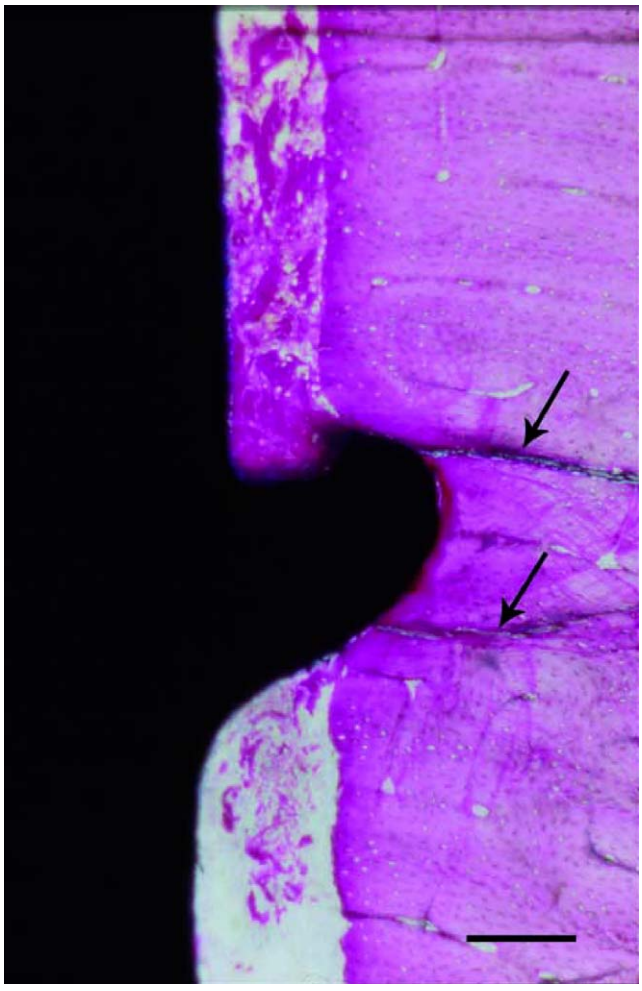

Fig. 7. Al-SLA implant 14 days after implantation. Light microscopy. The thread of the screw in close contact with the pre-existing bone appears to be deformed and bent. Fracture lines (arrows) are seen in the host bone at the tip of the thread. Fragments of host bone are detectable around the implant surface. Bar = 200 μm.

stability of an implant during fixation is considered to be a crucial factor for osseointegration and may be achieved through a close contact between the implant and bone cavity [20,30,31].

Another factor enhancing osseointegration during the early phases is the porosity of the implant surface. Some authors have demonstrated that pores exceeding 150 μm in diameter allow new bone ingrowth in plasma-sprayed-surfaced implants [32,33]. Microporous Ti oxide surfaces can rapidly promote bony ingrowth [34,35] and phosphate precipitation [36]. In accordance with previous histological studies sandblasted and acid-etched-surfaced implants have a higher percentage of bone-to-implant contact than plasma-sprayed implants, both at 3 months after surgery if unloaded and 12 months later after loading [19].

The results of this study showed new bone trabeculae with vascularised medullary spaces around unloaded

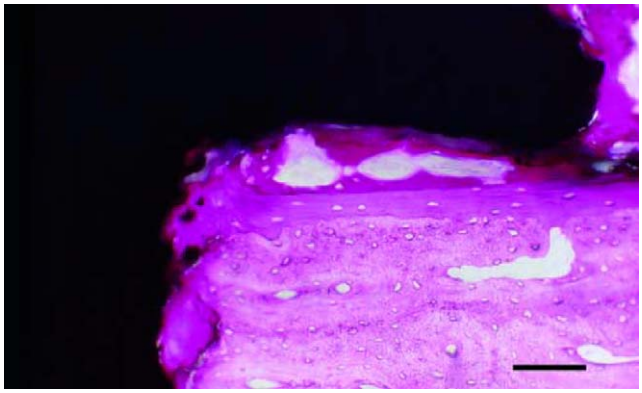

Fig. 8. TPS implant 14 days after implantation. Light microscopy. Ti granules detached from the implant surface are included in the newly formed peri-implant bone. Bar = 100 μm.

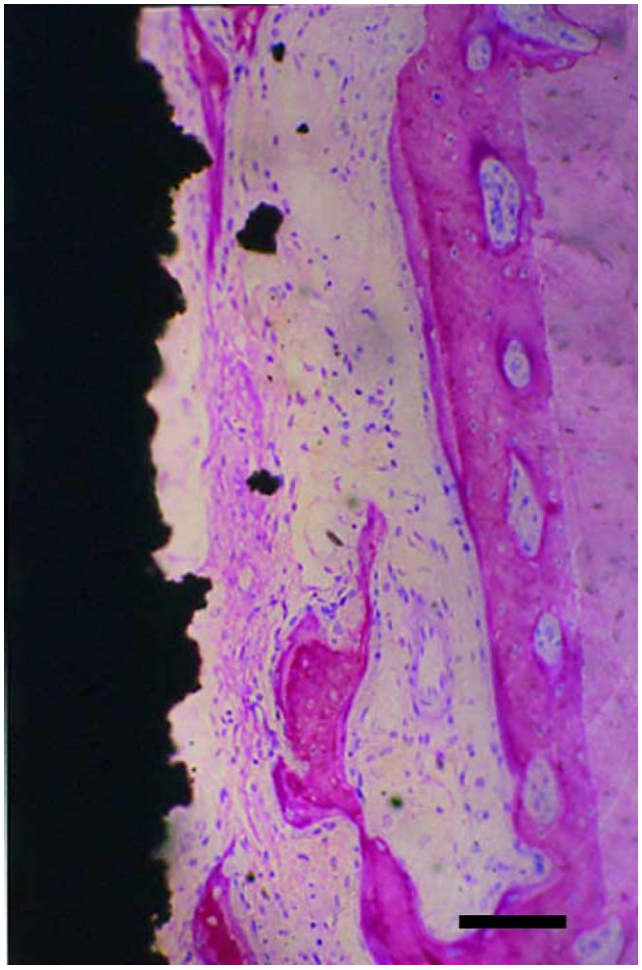

Fig. 9. TPS implant 14 days after implantation. Light microscopy. Ti granules detached from the implant surface are detectable in the highly vascularised medullary spaces of the periimplant tissues. Bar = 100 μm.

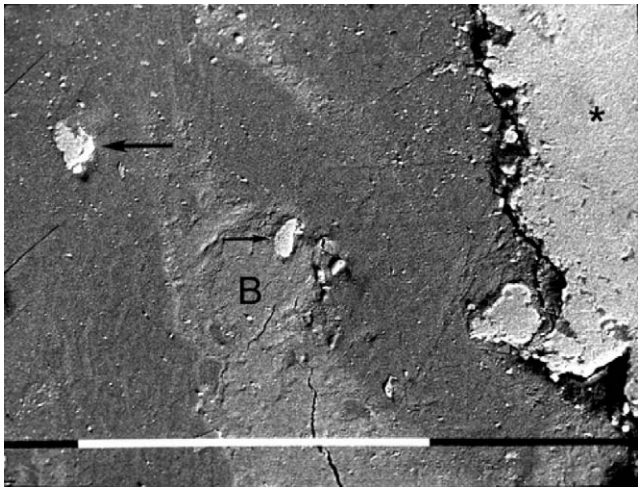

Fig. 10. TPS implant 14 days after implantation. Back-scattered SEM analysis. A Ti granule (high arrow) detached from the implant surface (*) is visible in the peri-implant medullary spaces. A Ti granule (low arrow) is surrounded by a new bone trabecula (B). Bar = 100 μm.

endosseous dental implants with different surfaces, 14 days after surgery. In addition, newly mineralised tissue was visible on all implant surfaces with various coatings, where gaps of 180–260 μm could be detected between the pre-existing bone and implant.

Neo-osteogenesis was also seen to occur around some isolated host bone chips resulting from the bur action during surgery. This host bone debris might be useful to enhance osseointegration on account of the osteoinductive property of the autologous fresh bone [41,42].

No new bone deposition was found in the areas where the implants had been inserted close to the osteotomy lines.

The current findings are consistent with those reported by other authors showing that osseointegration requires adequate spaces for bone remodelling, limited by the lack of implant mobility [37]. Moreover, a very tight contact of the implants with the bone cavities may be not fundamental for dental implantation as the injured pre-existing bone has been demonstrated to be located within 100 or even 500 μm from the cavity margin [37–40].

In a recent research [12] we demonstrated by back-scattered electron imaging and EDX analysis Ti granules, detached from unloaded TPS implant surface, to be present in the peri-implant tissues 12 weeks after surgery. Some authors suggested that Ti particles may be released from the implant surface during the preparation of the implant bed or implant insertion; they also observed metal debris in the peri-implant tissues, in other body organs [43,44], and in the regional lymph nodes [45,46].

In the present study a comparative analysis of the different implant surfaces showed that small Ti granules detached from the implant surface were visible at the implant–bone interface of the TPS implants both at zero time and 14 days after implantation. This finding indicates that such detachment occurs in the absence of loading and during the mechanical insertion of the implants, and is therefore related to the friction between the implant surface and host bone cavity. The friction of these implants is to be considered high since they were positioned in the femoral and tibial cortex with no screw tap. The final insertion torque resulted rather high (1.7 ± 0.1 Nm) in comparison with the peak insertion torques reported by Buser et al. [47]. Tight contact between implants and host bone, as well as friction between the two surfaces during implant insertion was also confirmed by the observation that some threads of the screws in close contact with the pre-existing bone appeared to be deformed. No material detachment was seen to occur in STi and SLA implant insertion. In agreement with our results Weingart et al. [45] detected Ti debris from TPS implant surfaces in beagle dogs 9 months after surgery. Frisken et al. [46] detected high levels of Ti from smooth, loose implants in the lungs and

regional lymph nodes of sheep 1, 4 and 8–12 weeks after surgery. Contrary to our data, Schliephake et al. [43] observed Ti particles in peri-implant tissues from smooth surfaced implants. This discrepancy might be explained by the different implant design, the site of insertion and the lack of a screw tap. If excessive friction is observed during the insertion of TPS implants, the porous coating surface presumably experiences physical microscopic damage with small Ti particle detachment during implant insertion: a "positive" Ti surface obtained for the apposition of particles, such as TPS, may be less resistant than a smooth STi or a "negative" Ti surface obtained for the detachment of Ti particles as happens with SLA implants.

These particles, detached during the implant insertion, are visible both in soft peri-implant tissue and newly formed bone. The loosening of particles during or after implantation endangers the safe application of very rough coatings [48]. Moreover, small metal particles inside the vascularised medullary spaces, as observed in our previous studies [12], may promote the Ti passive dissolution by increasing the surface extent of the metal in direct contact with the living body. Even if Ti particles may undergo cellular uptake and lysosomal degradation, the partially degraded Ti particles are still in peri-implant tissues 5–8 months after plates and screws insertion [44]. An excessive metal ion release has been shown "in vitro" to inhibit cell function and apatite formation [49,50].

5. Conclusions

Our research indicates that newly formed bone trabeculae are visible just 14 days after implantation in all the implant surfaces tested, where gaps of 180–260 μm are seen between the pre-existing bone and implants.

Data regarding Ti dispersion from the different implant surfaces demonstrate that the detachment of small metal particles from the TPS implant surface: (1) occurs in the early phases following implant fixation; (2) is not related to fretting against peri-implant tissues, since all implants were unloaded; (3) depends on the morphology of the implant surface; (4) may be related to the frictional force between the Ti coating surface and pre-existing bone during implant insertion.

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