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Residual aluminum oxide on the surface of titanium implants has no effect on osseointegration

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Abstract

The cleanliness of titanium dental implants surfaces is considered to be an important requirement for achieving osseointegration, and it has been hypothesized that the presence of inorganic contaminants could lead to lack of clinical success. Aluminum ions are suspected to impair bone formation by a possible competitive action to calcium. The objective of the present study was to describe the effects of residual aluminum oxide particles on the implant surface on the integration of titanium dental implants as compared to decontaminated implants in a rabbit experimental model. Threaded screw-shaped machined grade 3 c.p. titanium dental implants, produced with high-precision equipment, were used in this study. The implants were sandblasted with 100–120 μm Al_2O_3 particles at a 5 atm pressure for 1 min, then 24 implants (control implants) underwent ASTM F 86-68 decontamination process in an ultrasonic bath. The other 24 implants (test implants) were washed in saline solution for 15 min. Both test and control implants were air-dried and sterilized at 120°C for 30 min. After sterilization the implants were inserted into the tibiae (two test and two control implants in each rabbit). Twelve New Zealand white mature male rabbits were used in this study. The protocol of the study was approved by the Ethical Committee of our University. No complications or deaths occurred in the postoperative period. All animals were euthanized, with an overdose of intravenous pentobarbital, after 4 weeks. A total of 48 implants were retrieved. The images were analyzed for quantitation of percentage of surface covered by inorganic particles, bone–implant contact, multinucleated cells or osteoclasts in contact with the implant surface and multinucleated cells or osteoclasts found 3 mm from the implant surface. The differences in the percentages between the two groups have been evaluated with the analysis of variance. The implant surface covered by inorganic particles on test implants was significantly higher than that of control implants ($p = 0.0000$). No statistically significant differences were found in the bone–implant contact percentages of test and control implants ($p = 0.377$). No statistically significant differences were found in the number of multinucleated cells and osteoclasts in contact with the implant surface ($p = 0.304$), and at a distance of 3 mm from the implant surface ($p = 0.362$). In conclusion, our histological results do not provide evidence to support the hypothesis that residual aluminum oxide particles on the implant surface could affect the osseointegration of titanium dental implants.

Keywords: Aluminum oxide; Blasting procedure; Osseointegration; Surface cleanliness

1. Introduction

The integration of titanium implants in bone has been partly ascribed to the biocompatibility of the surface oxide layer [1]. The cleanliness of titanium dental implants surfaces is considered to be an important requirement for achieving osseointegration, and it has been hypothesized that the presence of inorganic contaminants could lead to lack of clinical success [2]. It has been reported that a small amount of fluorine contamination can dramatically alter the surface oxide of Ti implants during autoclaving [2]. It has been hypothesized that surface contamination may be released from the implant surface, enhancing and

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perpetuating the inflammatory response, altering the healing process and possibly provoking the dissolution of titanium [2]. Aluminum ions are suspected to impair bone formation by a possible competitive action to calcium [2–4]. This phenomenon was described around alumina coatings of cementless hip prosthetic stems. The presence of a consistent layer of decalcified bone tissue was demonstrated in continuity with and parallel to the prosthetic interface [5]; this demineralization has been attributed to a high concentration of aluminum ions [5–7]. It has been hypothesized that the impaired bone formation observed around Ti6Al4V implants, as compared to c.p. titanium, could be explained by the Al ion leakage [4]. Examination of a recovered titanium casting associated with tissue breakdown revealed the presence of embedded particles of alumina [8]. However, there are also experimental studies which do not indicate any significant differences in bone response between titanium and alloys [2]. The biological significance of the release of Al ions remains conjectural [2]. While unproven, the presence of aluminum is viewed with great concern as a possible causative agent in the observed tissue breakdown, and procedures avoiding aluminum blasting are recommended as a precautionary measure [8]. Moreover, lower removal torque values were found in alloys together with a tendency for c.p. titanium to have a higher percentage of bone–implant contact [9]. However, no controlled histological studies have been published testing this hypothesis. The objective of the present study was to describe the effects of residual aluminum oxide particles on the implant surface on the integration of titanium dental implants as compared to decontaminated implants in a rabbit experimental model.

2. Materials and methods

Threaded screw-shaped machined grade 3 c.p. titanium dental implants, produced with high-precision equipment, were used in this study. The implants were sandblasted with 100–120 μm Al_2O_3 particles at a 5 atm pressure for 1 min, then 24 implants (control implants) underwent ASTM F 86-68 decontamination process in an ultrasound bath in the following manner:

1. in distilled water for 15 min;
2. in 10% phosphoric acid (H_3PO_4) for 15 min;
3. in distilled water for 15 min;
4. in 70% nitric acid (HNO_3) for 15 min.

The other 24 implants (test implants) were washed in saline solution for 15 min. Both test and control implants were air-dried and sterilized at 120°C for 30 min. After sterilization the implants were inserted into the tibiae (two test and two control implants in each rabbit). It was decided to use the tibia as implant site for the simplicity of surgical access, and use only an experimental time (4 weeks) to limit the number of the animals used. The tibia is composed externally by cortical bone and internally by marrow spaces.

Twelve New Zealand white mature male rabbits were used in this study. The protocol of the study was approved by the Ethical Committee of our University. The rabbits were anesthetized with intramuscular injections of fluanizone (0,7 mg/kg b.wt.) and diazepam (1.5 mg/kg b.wt.), and local anesthesia was given using 1 ml of 2% lidocain/adrenalin solution. A skin incision with a periosteal flap was used to expose the bone. The preparation of the bone site was done with burs under generous saline irrigation. The implant insertion was performed by hand. The periosteum and fascia were sutured with catgut and the skin with silk. No complications or deaths occurred in the postoperative period. All animals were euthanized, with an overdose of intravenous pentobarbital, after 4 weeks. A total of 48 implants were retrieved.

Specimen processing: Implants and surrounding tissues were washed in saline solution and immediately fixed in 4% para-formaldehyde and 0.1% glutaraldehyde in 0.15 M cacodylate buffer at 4°C and pH 7.4, to be processed for histology. The specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy) [10]. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization the specimens were sectioned, along their longitudinal axis, with a high-precision diamond disc at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. A total of three slides were obtained for each implant. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Leitz Laborlux microscope (Leitz, Wetzlar, Germany). The histochemical analysis was done according to a previously published protocol [11]. The *histomorphometry* was carried out using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX). This optical system was associated with a digitizing pad (Matrix Vision GmbH) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc Milano, Italy). The images were analyzed for quantitation of percentage of surface covered by inorganic particles, bone–implant contact, multinucleated cells or osteoclasts in contact with the implant, and multinucleated or osteoclasts found 3 mm from the implant surface. Five additional implants for each group were analyzed under a Leo

scanning electron microscope (Zeiss, Hallbergmoos, Germany). Roughness measurements were performed for both types of implants, using a Mitutoyo Surftest 211 Profilometer (Mitutoyo Corporation, Tokyo, Japan): an average of three readings was performed for each surface.

Data analysis: The differences in the percentages of surface covered by inorganic particles in the two groups have been evaluated with the analysis of variance (ANOVA). The percentage of implant surface covered by inorganic particles was expressed as a mean $\pm$ standard deviation and standard error. The differences in the percentage of bone contact, multinucleated cells and osteoclasts in contact or near the implant surface between test and control implants were evaluated. The bone–implant contact percentage was expressed as the means $\pm$ standard deviation and standard error. Statistically significant differences were set at $p<0.05$.

3. Results

3.1. Surface characterization

3.1.1. Control implants

Some surface irregularities produced by the sandblasting were observed (Fig. 1). Residues of materials other than titanium were observed, and particles used for the sandblasting procedure were present (Fig. 2). The percentage of implant surface covered by inorganic particles was $0.9\pm0.4\%$. Surface roughness (Ra) was 2.11 μm.

3.1.2. Test implants

The surface was highly irregular with many small depressions and indentations (Fig. 3). A large quantity of inorganic particles was observed on the implants surface (Fig. 4). The percentage of implant surface covered by inorganic particles was $19.4\pm4.5\%$. Surface roughness (Ra) was 2.04 μm.

3.2. Light microscopy

3.2.1. Control implants

Newly formed bone was found in contact with the implant surface. Bone trabeculae were in close contact with the implant surface (Figs. 5 and 6). In some areas, newly formed blood vessels were observed. Some osteoblasts were actively secreting osteoid matrix directly on the implant surface, while, in other areas, osteoblasts were observed directly on the implant surface (Figs. 7 and 8). No lymphocytes or plasmacells were observed near the implant surface. A few positive ACP multinucleated giant cells, osteoclasts or macrophages were observed in the peri-implant bone tissue. The mean bone–implant contact percentage was $52.2\pm3.5\%$. The number of multinucleated cells and osteoclasts in contact with the implants was $3.3\pm0.8\%$,

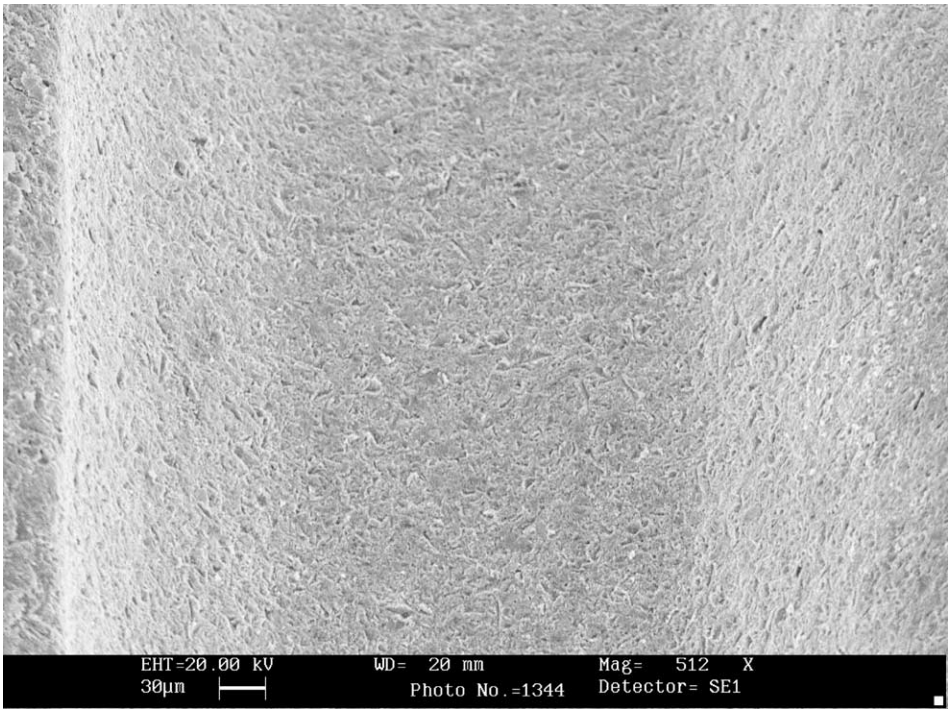

Fig. 1. Control implants. Irregularities produced by the sandblasting are present.

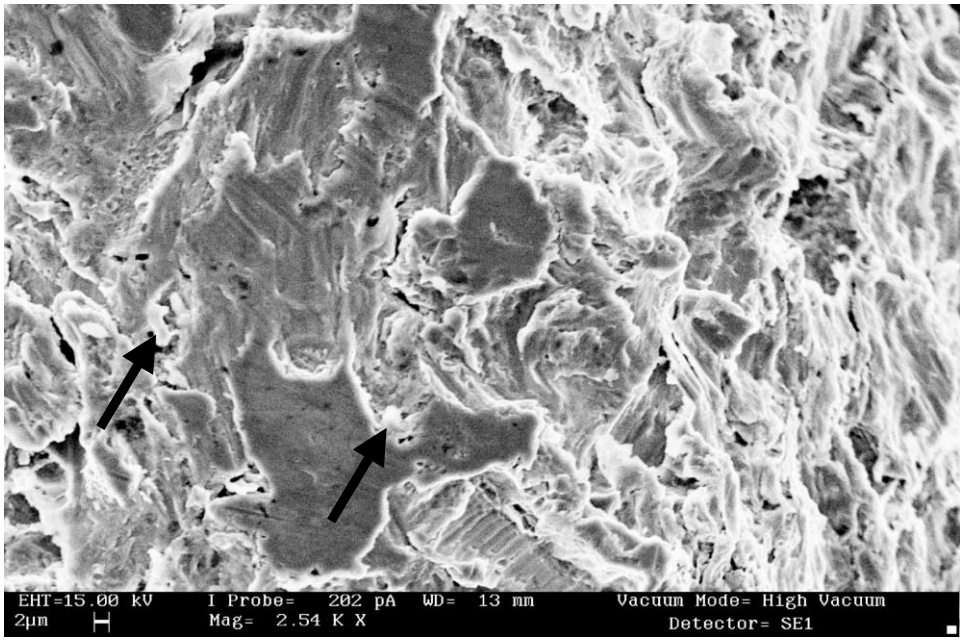

Fig. 2. Control implants. A few residues of particles (arrows) used for the sandblasting procedure are present.

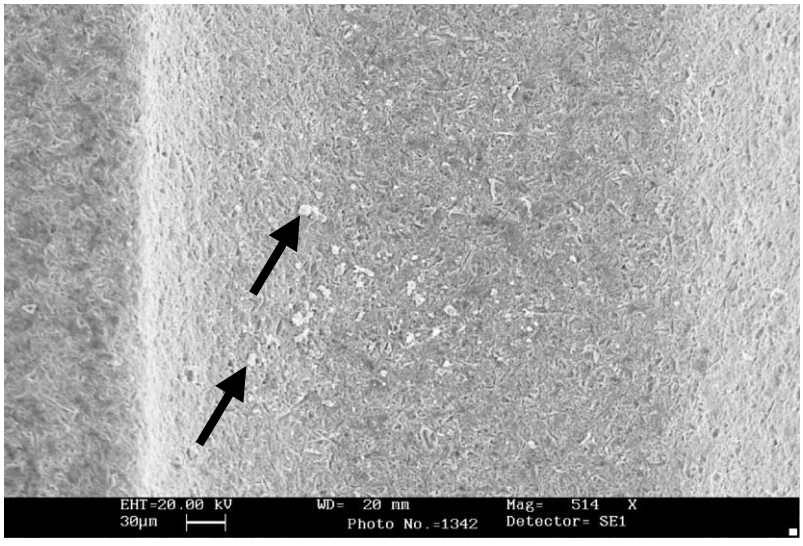

Fig. 3. Test implants. Some surface irregularities produced by the sandblasting are present. Residues of materials other than titanium (arrows) are observed.

while the number of these cells evaluated at a distance of 3 mm from the implant surface was $5.2 \pm 4.3\%$.

3.2.2. *Test implants*

Mature mineralized bone and, only in a few areas, not yet mineralized osteoid matrix were present at the interface in the cortical region (Fig. 9). Mature bone and marrow spaces were present in other areas of the interface (Fig. 10). Only in a few portions of the interface, actively secreting osteoblasts were observed in marrow spaces (Figs. 11 and 12). Bone peri-implant trabeculae were thick. No lymphocytes or plasmacells were observed near the implants surface. A few positive ACP multinucleated giant cells, macrophages or osteoclasts were observed in the peri-implant bone tissue. The mean bone–implant contact percentage was $53.1 \pm 2.9\%$. The number of multinucleated cells and osteoclasts in contact with the implants was $3.1 \pm 0.5\%$, while the number of these cells evaluated at a distance of 3 mm from the implant surface was $6.1 \pm 2.1\%$.

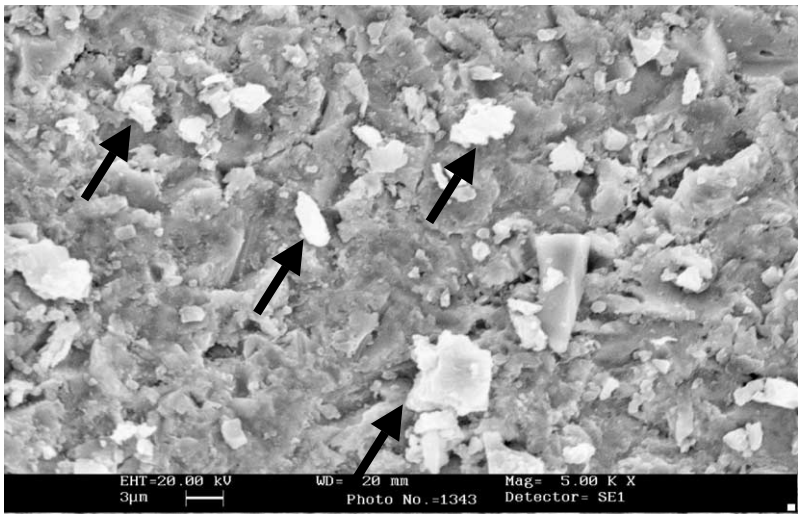

Fig. 4. Test implants. A large quantity of inorganic particles (arrows) is observed on the implant surface.

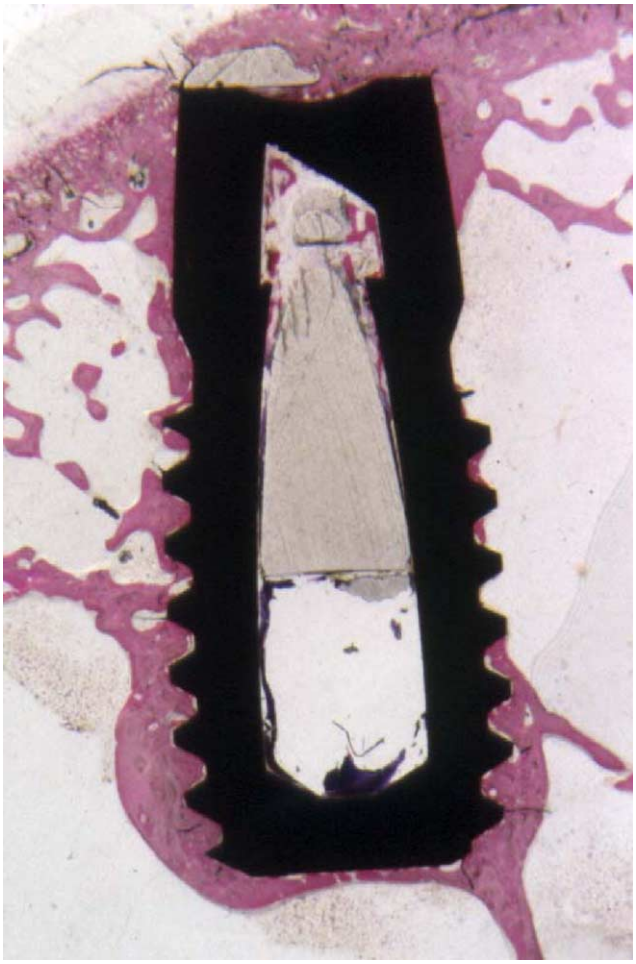

Fig. 5. Control implants. Mature compact bone is present in close contact with the implant surface. Toluidine blue and acid fuchsin 20 ×.

3.2.3. Statistical evaluation

The implant surface covered by inorganic particles on test implants was significantly higher than that of control implants ($p = 0.0000$) (Table 1). No statistically significant differences were found in the bone–implant contact percentages of test and control implants ($p = 0.377$) (Table 2). No statistically significant differences were found in the number of multinucleated cells and osteoclasts in contact with the implant surface ($p = 0.304$) (Table 3), and at a distance of 3 mm from the implant surface ($p = 0.362$) (Table 4).

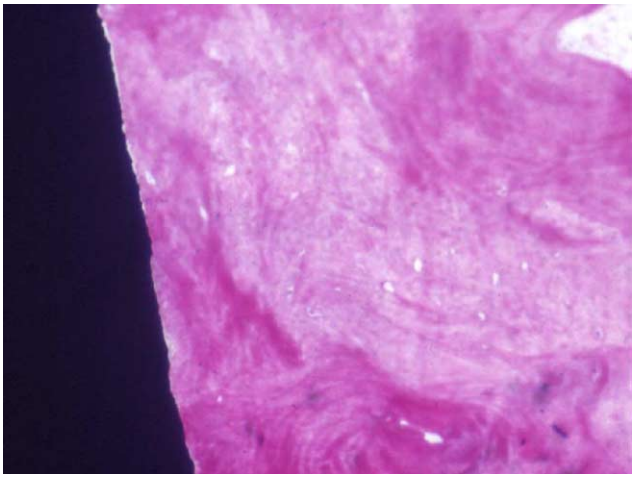

Fig. 6. Control implants. No gaps between implant and bone were observed. Toluidine blue and acid fuchsin 400 ×.

4. Discussion

The role of implant surface contamination in implant failures is not yet well understood [2]. Implant surface contaminants may be released from the surface and they may elicit an inflammatory response [2]. Blasting the implant surface with particles other than the implant itself may change the surface composition and the

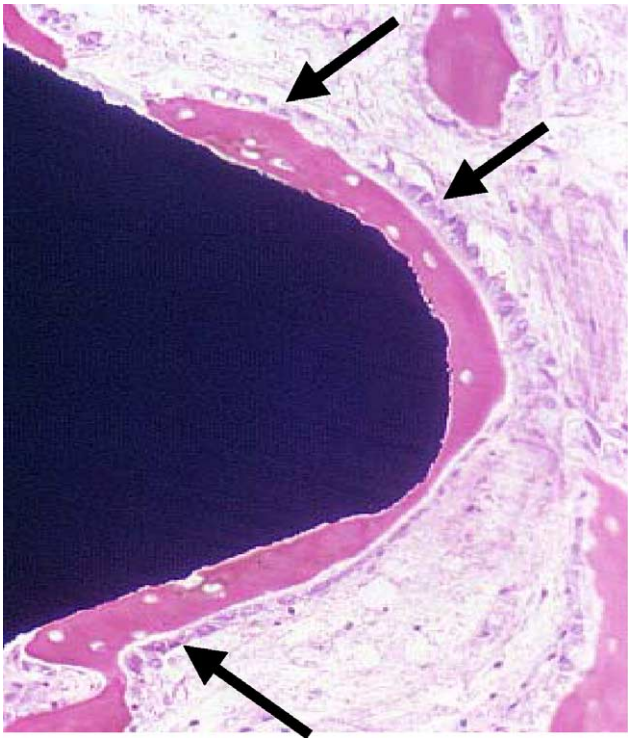

Fig. 7. Control implants. Osteoblast activity (arrows) is present in marrow spaces. Toluidine blue and acid fuchsin 200 × .

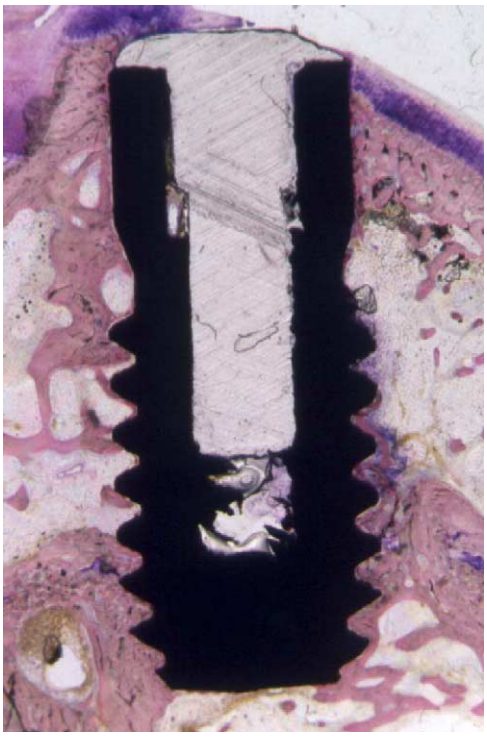

Fig. 9. Test implants. A mature compact bone is present around the implant perimeter. Toluidine blue and acid fuchsin 20 × .

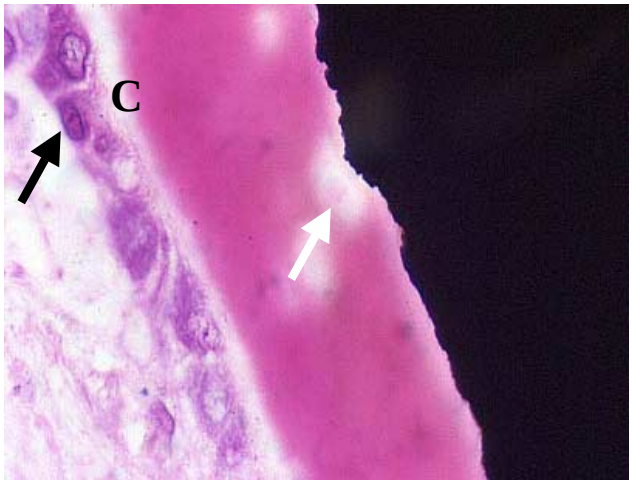

Fig. 8. Control implants. The bone and osteocyte lacunae (white arrow) are in close contact with the implant surface. A line of cuboidal-shaped osteoblasts (black arrow) and osteoid matrix (O) are visible around the implant perimeter. Toluidine blue and acid fuchsin 1000 × .

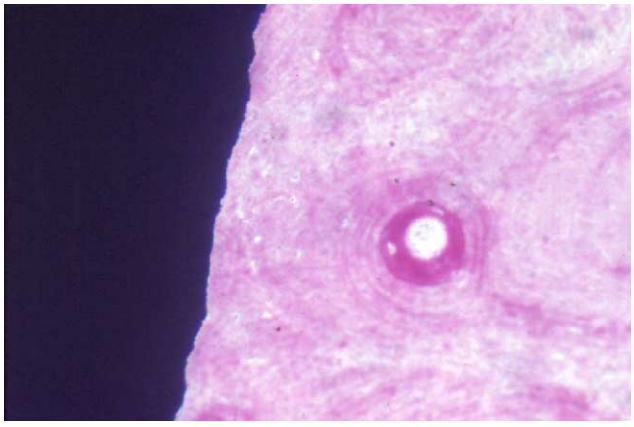

Fig. 10. Test implants. No gaps between implant and mature bone are observed. Toluidine blue and acid fuchsin 400 × .

implant biocompatibility [12]. Abrasive blasting increases the surface roughness, and increases metal surface reactivity [12]. With the use of a blasting material like Al_2O_3, a potential risk of presence of remnants of blasting particles with dissolution of Al ions into the host tissue cannot be excluded [12]. It has been reported that Al ions may inhibit normal differentiation of bone marrow stromal cells and normal bone deposition and mineralization [13–15], and aluminum has been shown to induce net calcium efflux from cultured bone [16]. Moreover, aluminum may compete with calcium in the healing implant bed, and aluminum has been shown to accumulate at the mineralization front and in the osteoid matrix itself [3]. Nimb et al. [3] found, in a study in dogs, that aluminum inhibited the formation of calcium phosphate crystals and the growth of poorly crystallized hydroxyapatite. Completely different results have, however, been published on the effects of aluminum in bone. Quarles et al. [17] reported that aluminum administration to beagle dogs stimulated

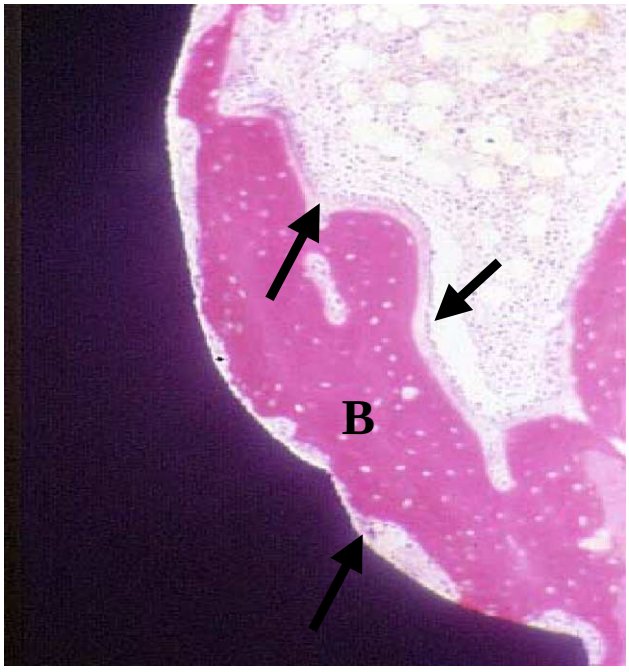

Fig. 11. Test implants. New bone (B) and osteoblasts (arrows) produce osteoid matrix near the implant surface. No lymphocytes or plasmacells are observed near the implant surface. Toluidine blue and acid fuchsin 200 ×.

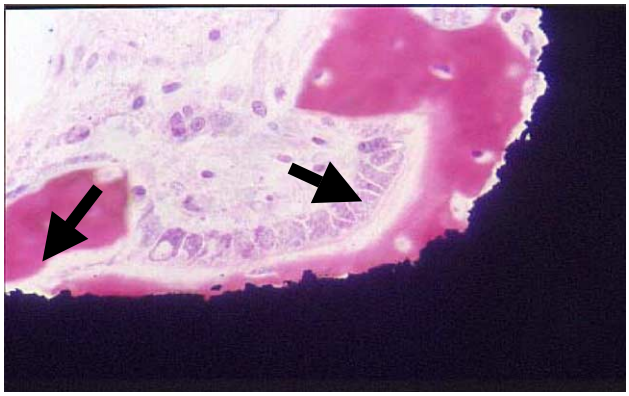

Fig. 12. Test implants. In one area, osteoblasts produce osteoid matrix directly on the implant surface. No lymphocytes or plasmacells are observed near the implant surface. Toluidine blue and acid fuchsin 100X.

Table 1
Statistical evaluation of percentage implant surface covered by inorganic material

	Mean	S.D.	S.E.	*p*-Value
Control implant	0.9	0.4	0.0817	0.0000[a]
Test implant	19.4	4.5	0. 919	

[a] Significant at 95% (according to the ANOVA test).

uncoupled bone formation with an increase in trabecular bone volume. Also Lau et al. [18] reported that Al ions might stimulate bone formation in vitro. Feighan

Table 2
Statistical evaluation of percentage of bone–implant contact

	Mean	S.D.	S.E.	*p*-Value
Control implant	52.2	3.5	0.714	0.337[a]
Test implant	53.1	2.9	0.592	

[a] Non-significant.

Table 3
Statistical evaluation of number of multinucleated cells and osteoclasts in contact with implants

	Mean	S.D.	S.E.	*p*-Value
Control implant	3.3	0.8	0.1633	0.304[a]
Test implant	3.1	0.5	0.102	

[a] Non-significant.

Table 4
Statistical evaluation of number of multinucleated cells and osteoclasts found 3 mm from the implant surface

	Mean	S.D.	S.E.	*p*-Value
Control implant	5.2	4.3	0.888	0.362[a]
Test implant	6.1	2.1	0.429	

[a] Non-significant.

et al. [19] showed that Al_2O_3 blasted implants presented woven and lamellar bone in direct apposition to the implant surface, and this fact was evidence of active bone formation toward the implants. To overcome the potential risks of surface contamination, blasting particles of different materials have been used. For example, with the use of TiO_2 particles no foreign elements are added to the surface [12]. Wennerberg et al. [12] published a study in rabbit, using implants blasted with 25 μm particles of TiO_2 and Al_2O_3. These authors found no statistically significant differences between the implants in the bone–implant contact percentages and in the removal torque values [12]. They concluded that no differences were found between the implants blasted with the same size of the particles but using different blasting materials and that they could not detect any negative effect of the aluminum [12]. These results were confirmed in other studies [20,21]. In a previous study from our laboratory we found that no untoward effects on peri-implant bone regeneration were present due to Al_2O_3 blasting procedure [22]. These results are in contrast with those found with Ti6Al4V alloy implants, where differences in bone–implant contact percentages or removal torque values were present [20]. These results can be explained by the fact that in the alloy there is the potential of a continuous release of Al ions into the tissues, while in the Al_2O_3 blasted implants only a transient and limited release of Al ions is possible [20]. Moreover, the 25 μm TiO_2 and Al_2O_3 blasted surfaces

showed very similar surface structures, quantitatively as well as qualitatively [20]. The Al_2O_3 blasted implants showed significantly more aluminum on the surface compared to the machined implants but, on the other hand, the composition of the implants surface was found to be similar between the blasted and unblasted implants [20]. The observed presence of Al on the surface indicates that a transfer of the blasting material onto the metal surface has taken place [20]. Under SEM, a few particles, probably arising from the blasting material, were observed [20]. A major part of the Al signal is, however, probably due to monolayer amounts of Al produced by adhesive wear [20]. The detected amounts of Al are much higher on Ti6Al4V [20].

Surface contaminants seem not to play an important role in the process of implant failures [2]. In a study evaluating the surface of failed oral titanium implants, Esposito et al. [23] found that no material related causes for the failures of these implants could be found. The surface of titanium implants consists of a thin (2-6 nm) oxide (mainly TiO_2) covered by a carbon-dominated contamination layer and trace amounts of N, Ca, P, Cl, S, Na and Si [24]. Surface analyses of the chemical composition of various dental implant systems showed various degree of contamination on their surfaces [25]. An experimental investigation did not demonstrate any statistical difference in bony contact between biologically contaminated titanium implants and non-contaminated controls [26]. All implant materials release charged particles/ions to some degree as result of corrosion and/or wear, due to the action of highly aggressive body fluids and strong mechanical stresses [27]. After a comprehensive review of the literature, it was concluded that most statements about "inert biomaterials" in part relied on analytical investigations with insufficient sensitivity. However, when analyzing studies dealing with trace elements in human body fluids and tissues, the reader should be aware that major unsuspected methodological errors may occur during the manipulation of the samples, which can invalidate the reliability of the results [28]. However, only low or very low levels of trace elements have been found in various organs and in blood when accurate and controlled methodologies were employed [29–32]. So far, there is no evidence to support any toxic effects due to wear particles or metal ion release. However, more studies, specifically addressing this matter are required. Animal models are essential in providing phenomenological information on biological reaction to implants inserted in bone [22]. In the present study the authors wanted to evaluate the degree of osseointegration after 30 days. In fact, previous researches had shown that the surface characteristics were important in influencing the bone–implant contact percentages and statistically significant differences were observed after 30 days in different implant surfaces [22]. The results of the present work show that bone–implant contact is similar in test and control implants. The biochemical and biological function of osteoblasts seemed to be preserved in the presence of the Al_2O_3 residual particles. After one month, no substantial inflammatory response was shown. If a substantial inflammatory response occurred at an earlier time point, it did not adversely affect bone–implant integration. Furthermore, this model would assess the effects of both aluminum oxide particles on the implant surface as well as particles which may have become dislodged and/or sheared upon placement in bone.

In conclusion, our histological results do not provide evidence to support the hypothesis that the presence of residual blasting aluminum particles on the surface of dental implants could affect the osseointegration of titanium dental implants.

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