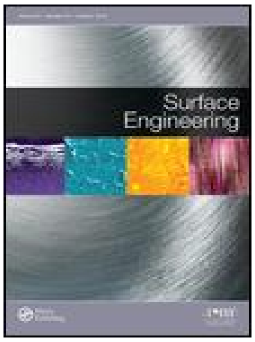

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Osseointegration evaluation of plasma nitrided titanium implants

C. L. B. Guerra Neto*, M. A. M. da Silva and C. Alves, Jr

There is a need for new techniques that modify metallic implant surfaces, resulting in good bone response for low density areas. Among the different techniques, plasma nitriding is being applied in biomedicine with excellent results. However, its use in titanium dental implants is very limited owing to high process temperatures (between 700 and 800°C) that result in distortions. In order to solve this problem, the authors used a new plasma method with a hollow cathode discharge configuration. Ti grade II dental implants were nitrided in a 20% N_2–H_2 atmosphere at a temperature of 450°C, 150 Pa (1·5 mbar), for 1 h and inserted into rabbit tibias to assess osseointegration. The results showed that plasma nitriding caused a significant change in the surface texture of the nitrided implants and greater removal torque than that of the control implants.

Keywords: Osseointegration, Dental implants, Surface modifications, Plasma nitriding, Biomaterials

Introduction

Since the groundbreaking studies of Branemark *et al.*[1,2] and Schroeder *et al.*,[3] osseointegrated implants have become a reliable dental restoration tool.[4,5] They were developed to follow a strict surgical protocol that enables the treatment of partial or total tooth loss and continue to be universally accepted.[4–6] Although the implant technique is recognised as a reliable option for restoring tooth loss, it has been shown that certain surfaces promote greater and faster bone contact in the healing stage and longer lasting durability, which could certainly contribute to perfecting the procedure.[5] These titanium surface characteristics could also, for example, enable the placement of immediately functioning implants in previously contraindicated areas and widen the gamut of current applications. The need to define a metallic implant surface compatible with good bone response is important in basic implant research, especially in the search of solutions for areas of low bone density.[7]

Once any implant comes in contact with the biological environment, it is characterised by dynamic changes in its surface properties. These changes involve a series of reactions that occur between the biological and biomaterial environment[8,9] which form a ‘conditioning film’ that modulates the cell responses of the host.[10]

Some studies report that wettability and surface energy play an important role in protein adsorption by increasing the formation of osteoblast focal adhesions[11–13] on the surface of the implant. A surface topography was described by Rupp (2004), where spatial and hybrid roughness parameters increased dynamic wettability of the surface. Considering that roughness and wettability interfere with protein adsorption processes, cells may be strongly influenced by both, if they reach the implant surface.[11]

It has been observed that faster osseointegration can be achieved by texturising the implant surface.[14–16] This may be due to the osseointegration mechanism described by Kasemo in 2002. Based on this mechanism, the real precursor of osseointegration is surface wettability by the biological liquid rather than roughness, as was previously believed.[15] Water molecules are the first to reach the titanium surface. Surface wettability influences proteins, molecules and cells that arrive a little later.[15,16]

Many studies have attempted to find surfaces that provide rapid osseointegration. Accordingly, surfaces have been modified by numerous processes[17] involving mechanical, chemical and physical surface treatment methods, resulting in various degrees of texture.[18] It is in this sense that the authors propose the present study. The aim is to find a surface that satisfies this need. The authors chose plasma nitriding for its excellent mechanical properties, chemical stability and biocompatibility[19] when applied to titanium. Its use in hip, knee, shoulder and ankle implants has led to increased resistance to abrasion and reduced bacterial colonisation, when compared to other clinically used implant surfaces.[20] However, a limitation of using nitriding in dental implants is the high process temperature (between 700 and 800°C), which may cause distortions in the implant due to its geometry and dimensional precision.[21] An alternative for solving this problem is a treatment using a hollow discharge cathode (HCD) configuration. This method consists of nitriding the implant part in a highly ionised atmosphere. A high density of ions means greater surface bombardment, causing high energetic incidence.[22] This electrode configuration is used to

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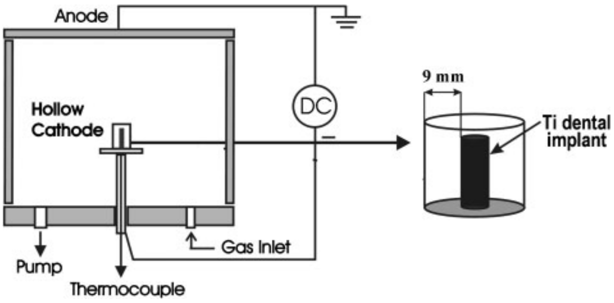
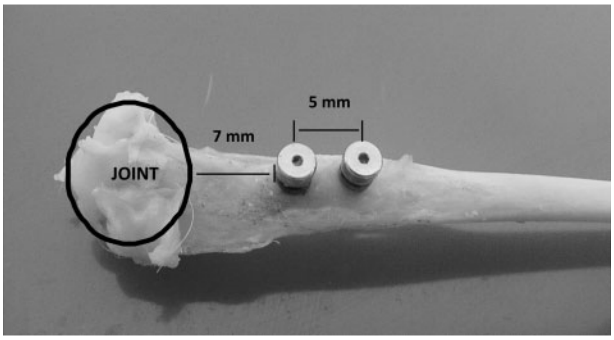


1 Experimental device used for plasma nitriding in implants



2 Location of rabbit tibia implants

increase superficial damage and obtain a thermal gradient produced by the increased collision and ionisation rate.[23] In this study, Ti surfaces were nitrided by plasma using a HCD technique aimed at dental implant applications. Important aspects such as osseointegration, torque removal and texture were observed.

Materials and methods

The authors used 24 Ti grade II implants (length=6·25 mm, diameter=3·2 mm) supplied by A.S. Technology (Titanium Fix, São Paulo, Brazil). They were inserted into six adult white New Zealand rabbits, weighing between 2·0 and 2·5 kg. Of these 24 implants, 12 were plasma nitrided by HCD, in a 20% N_2–H_2 atmosphere, at a pressure of 150 Pa (1·5 mbar) and temperature of 450°C for 1 hour and 12 remained untreated (control). These nitriding conditions were based on previous systematic studies performed with pure Ti grade II samples under varying pressure, treatment time and temperature.[23]

For nitriding, the implants were cleaned before the process as follows: enzymatic detergent, alcohol and bidistilled water for 10 min in ultrasound.

The experimental apparatus used for plasma nitriding consisted of a vacuum chamber containing an anode and a hollow cathode (all the metallic parts were made of stainless steel coated inside with commercially pure Ti), into which the implants were placed and centred. The HCD conditions were satisfied in the space between the inner wall and the implant surface, fixed at 9 mm, all of which are under the same negative potential. The gas flow mixture was maintained constant at 16 sccm for all the experiments. The temperature was measured with a chromel-alumel thermocouple inserted in the sample port and controlled by the continuous variation among electrodes (Fig. 1).

Following treatment, both the nitrided and untreated implants were submitted to a strict 10 min cleaning protocol in ultrasound using a DEIV 3E solution to remove fats, proteins and carbohydrates. In addition, they were washed in absolute alcohol for 10 min and in distilled water for a further 10 min, both in ultrasound. Each step was repeated twice. The samples were placed in a drier for 1 min and conditioned in hydrogen peroxide sterilisation packaging.

After sterilisation the implants were inserted approximately 7 mm from the metaphysic proximal of the tibias (Fig. 2). The surgeries were performed under a strict surgical protocol. The animals were selected for surgery and treated according to established norms for experiments with animals.

The animals were subcutaneously sedated with 0·5 mg mL^{-1} atropine sulphate, an anticholinergic, at a dose of 0·1 mg kg^{-1}. After 10 min, a 20 mg mL^{-1} dose of Dorsipec (Xylazine) sedative was administered as well as an intramuscular injection of 3 mg kg^{-1} neuroleptic painkiller in the semitendinous muscle. The tibia region of the sedated animals was trichotomised, which was followed by antisepsis with PVPI (Povidine). The animals were placed under general anesthesia with a 30 mg kg^{-1} intramuscular dose of 50 mg mL^{-1} Vetanarcol (Ketamin Chloride) dissociative anesthetic, and submitted to surgery after endovenous administration of a 7·5 mg kg^{-1} dose of Vetanarcol. The animals underwent a six hour fast before the surgery. Local anesthesia was then performed with approximately 0·9 mL of 2% lidocaine in the medial side of each tibia, where the incision was made.

After the incision, the skin tissue was retracted with Kelly hemostatic forceps until total access to the tibia was obtained. Implant cavity preparation was initiated using a BLM 600 Plus driller with 1900 rev min^{-1} and 45 N cm of torque, externally irrigated with a 20 mL syringe of saline solution. The drill sequence followed the step by step recommendations of the company that manufactured the implants. At this moment the implants were placed on the surgical cavity with the help of a ratchet wrench (Fig. 3).

The implant was then closed with a cover screw and sutured. Finally, the rabbits were identified with an electric scalpel and administered post-operatory medication. This procedure was repeated with all the rabbits. All the surgeries were carried out on the same day at the surgical centre of the Clinica de Assistência Veterenária (Veterinarian Assistance Clinic). One rabbit was killed at 15 days post-operatory and the four implants inserted in it were observed under a scanning electron microscope (SEM) to evaluate bone/implant interface. These implants were removed by breaking the tibias. Another animal was killed at 60 days, where the implants of one tibia were removed by torque and the other tibia was transversally cut to observe bone healing under optical microscope. The other four rabbits were killed at 90 days. Of these, three were used for torque removal and one was observed for bone healing under a SEM. The torque removals were performed with a TQ-8800 digital torque meter, with a range between 0·1 and 147·1 N cm and resolution of 0·1 N cm.

The implants and surrounding tissue were removed in blocks, and identified. The samples were moulded and metallographically prepared using 500–2400 grit silicon carbide sandpaper until the first thread of the screw

a opening of surgical cavity; *b* inserting implant into cavity; *c* positioning implants in tibia
3 Surgical insertion sequence of implants

appeared. The finely polished surface of the sample was set up on a glass slide (50 × 25 mm) and glued with Araldite glue for 10 min. The slide and sample were placed under weight and dried under pressure for 10 h.

The sample and the slide were connected to a vacuum support in a high precision microtome (Ingram-Ward Petrographic Thin Section Cut-off Saw Models 135 and 137U). The cut started at the head of the implant, using a diamond disk. The samples were then polished with 500, 1200 and 2400 grit sandpaper to a thickness of 40 μm. The thickness of the section was measured by caliper at regular intervals. The samples were then observed under optical microscope using Image Pro Plus software.

Results and discussion

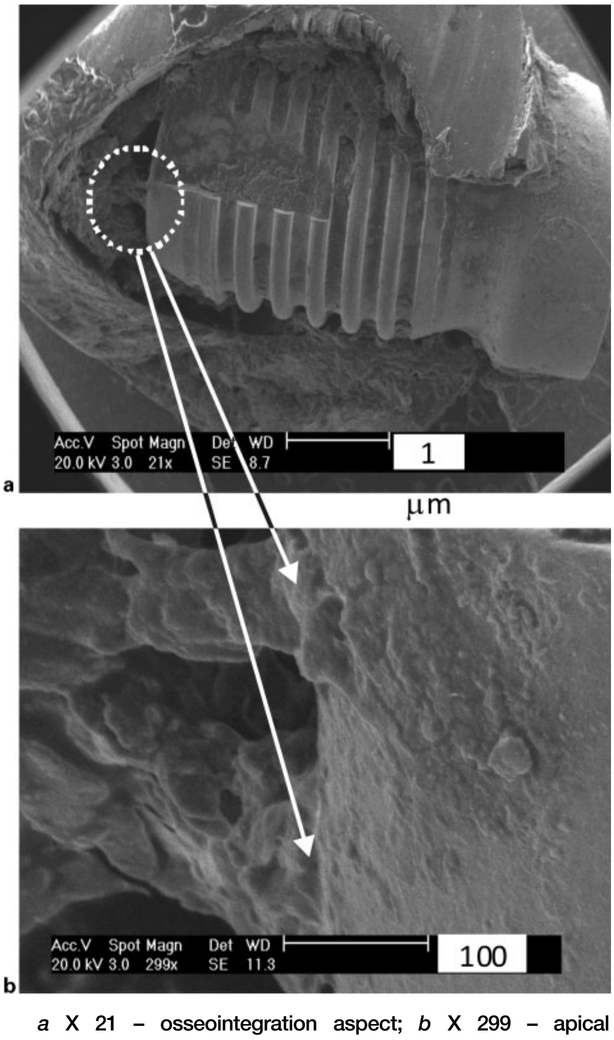
Texture and crystalline phase are important aspect for assessing the contact between two surfaces. If two surfaces have the same chemical characteristics, it means that the tension between the two surfaces will be greater. The examination under a SEM of the surface of nitrided implants (Fig. 4) shows uniform texture over their entire length. At 860X superficial uniformity and occlusions can be seen. Surface characterisation by XRD shows tipically δ-tiN e ε-ti$_2$N.[24]

Nitrided surfaces favour wettability, which enables increased contact between the two surfaces. At 13 000 X when the authors compare with an untreated implant surface, it can be observed that the latter is not smooth, as has been described in the literature, has a heterogeneous surface with microfissures and machining defects (Fig. 5).

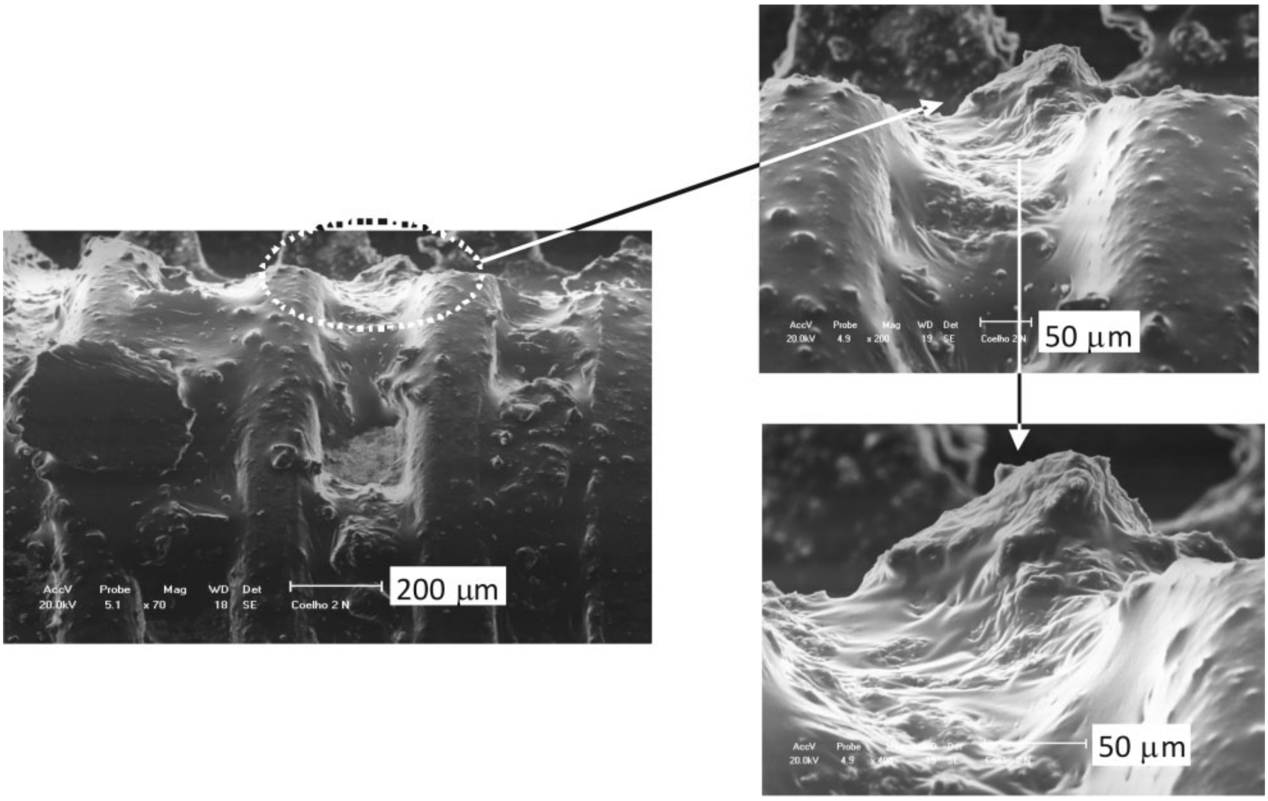
The results observed under a SEM at 15 days were good. Figure 6 shows a close relation between bone formation and the nitrided implant, despite only 15 days of healing. It was impossible to identify the bone–implant border. The bone surrounding the nitrided implant seems to be strongly incorporated into it, showing final osseointegration at some points, even though studies report that osseointegration starts only after three weeks.[25] After three weeks, Johansson *et al.*[26] found a fibrous interface between Ti and the bone, whereas the results clearly show osseointegration in less than 15 days.

The implants at 60 days were examined under an optical microscope and submitted to torque removal. Optical microscope examination reveals a band of neoformed bone surrounding the entire implant surface, which clearly shows the three structures (implant, neoformed bone and mature bone) (Fig. 7). Torque removal values were 30·95 N cm for the nitrided implants and 13·75 N cm for the untreated implants.

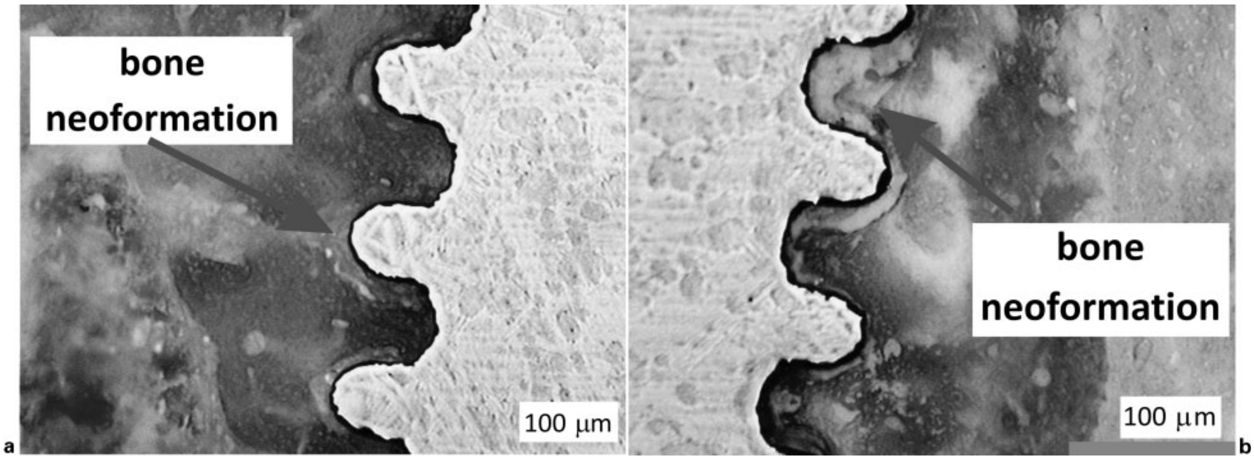
The torque removal values of the nitrided implants at 90 days were higher than those obtained by untreated implants, with a mean of 53·2 and 31·2 N cm for nitrided and untreated implants respectively. These means were the same as those found by Johansson *et al.*[26] in 1988 at 90 days. Cho and Park[27] found a slightly higher result, but his implants were inserted bicortically, different from the nitrided implants in this



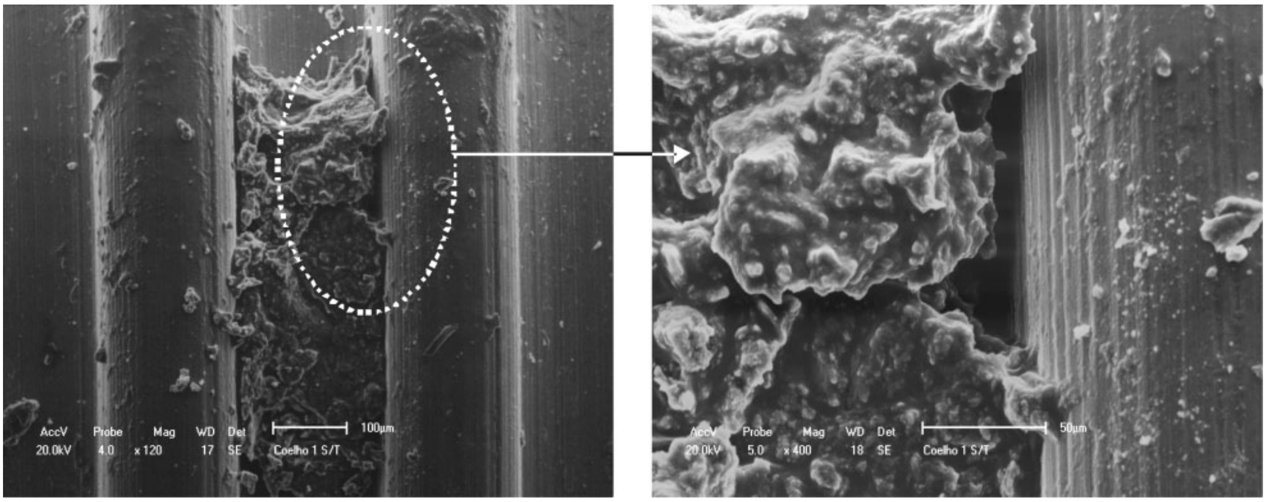
a X 21 – osseointegration aspect; *b* X 299 – apical region
4 Image (SEM) for nitrided implants before 15 days



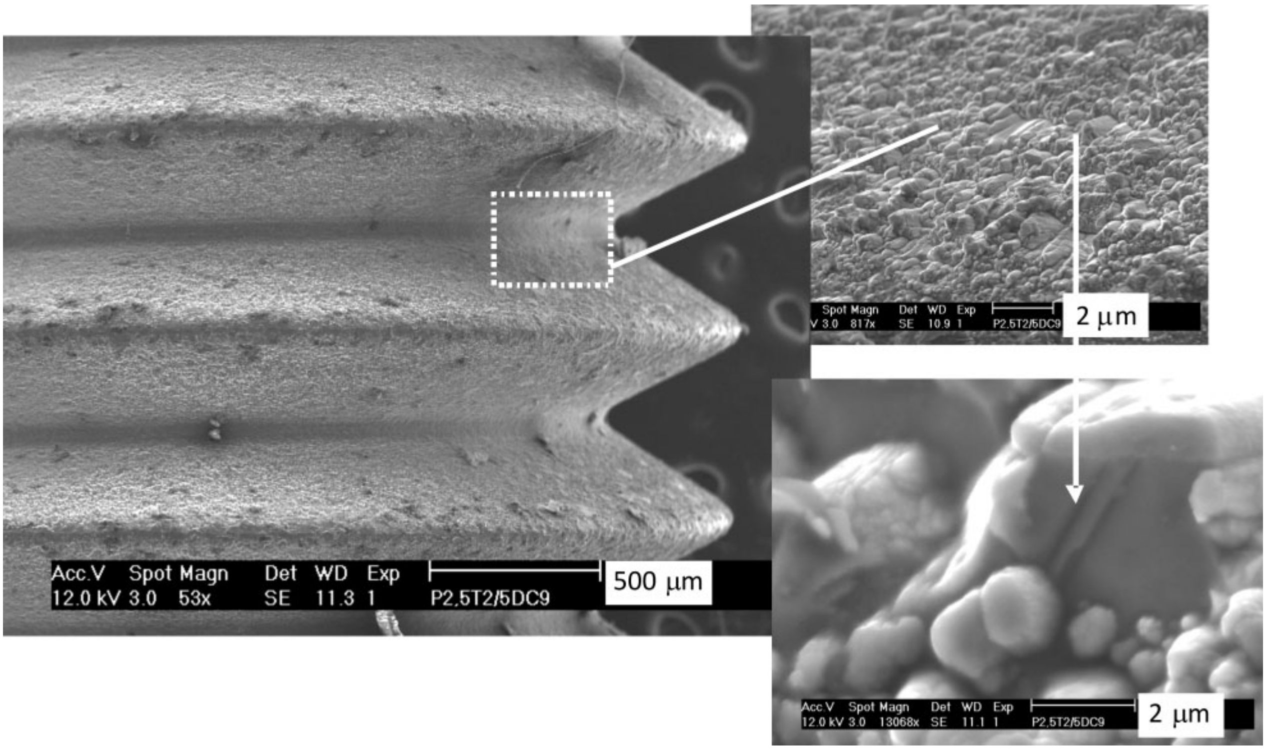
5 Image (SEM) for nitrided implant to draw back by torque before 60 days – details for bone deposition around implant surface



6 Image (SEM) of *a* nitrided implant at 15 days and *b* close-up of apical region



7 Image (SEM) untreated implant to draw back by torque before 60 days – details for poor interface bone/implant



8 Image (SEM) – morphology of nitrided implant surface

study inserted into only one cortex. This shows that, even when inserted into one cortex, osseointegration of the nitrided implant was greater than that of the acid-etched implant. Comparing with the study by Karacs *et al.*[28], the authors observed that the removal torque of nitrided implants was higher than that of acid and laser etched surfaces.

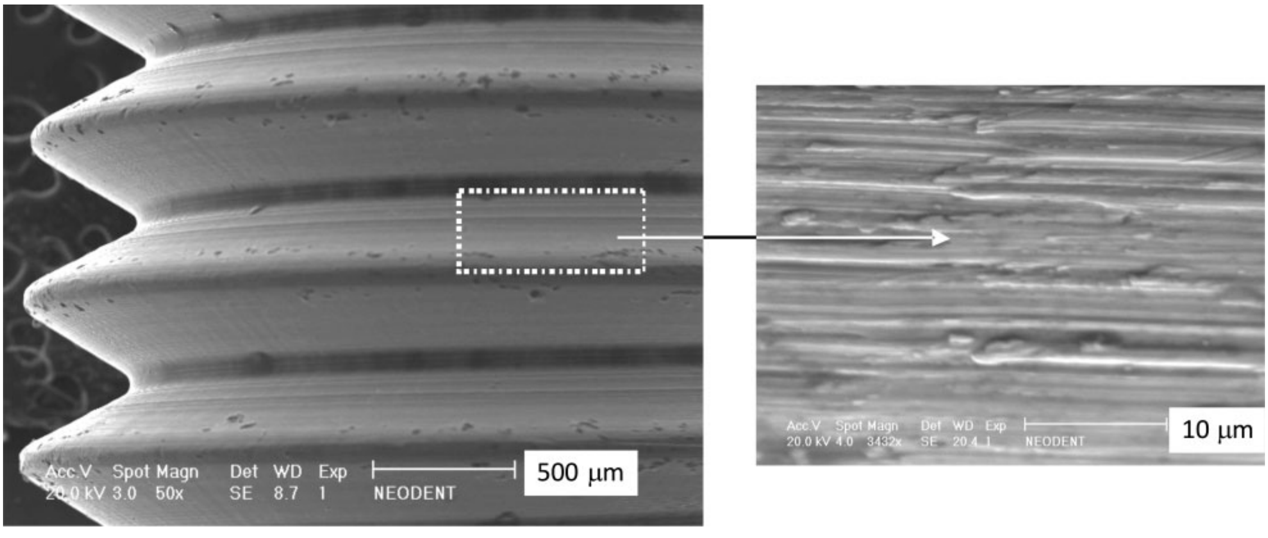
After torque removal at 90 days, the nitrided and untreated implants were examined under a SEM. The authors observed a complete recovering of the threads and filling of the grooves. The authors also found a much higher bone–implant contact index than that of untreated implants (Fig. 8).

The untreated implants show a small amount of bone adhesion with fissures and few bone–implant contact points. Figure 9 shows the bone flaws that explain their lower removal torque.

Conclusions

The following conclusions can be drawn based on the *in vitro* and *in vivo* evaluation of plasma nitriding in titanium substrates.

1. Plasma nitrided implants using HCD produce a non-toxic surface, biocompatible and suitable for biological use.
2. Nitrided HCD surfaces have a uniform texture with microcavities.
3. Mean torque removal values of nitrided implants were double those of untreated implants (control).



9 Image (SEM) – morphology of untreated implant surface

4. The osseointegrated perimeter for the nitrided implants was greater than that for untreated implants (control).

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