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Osseointegration of Titanium Dental Implants Modified by Thermal Treatment: Preliminary Data in a Rabbit Model

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Abstract

Background: In the field of implant dentistry, titanium alloy surfaces represent the gold standard for their biocompatibility, physical and mechanical characteristics. Differences in fixture, microtexture and the titanium oxide layer formation on the surface are important for conditioning and improving the bone response around dental implants.

Purpose: The aim of this study is to evaluate the influence of a thermal treatment of a Titanium implant surfaces and the bone healing response *in vivo*.

Methods: A total of 4 titanium implants were positioned in a split model experiment; 2 fixtures were thermally treated and inserted in rabbit femoral knee test sites, and 2 fixtures were inserted in the control sites. The samples were histologically and histomorphometrically evaluated at 8 weeks.

Results: No statistically significant differences (p = 0.156).were present histologically in the percentages of bone-implant contact (BIC) between the Test Group (BIC= 68.83 ± 6.8%) and Control Group (BIC = 54 ± 5%).

Conclusions: The outcome of the present preliminary study indicates a novel approach to improving bone healing around titanium implants. Furthermore, more studies with increased sampling number are necessary to validate the results of the surface thermal treatment and their effect on novel bone formation.

Keywords: *Flame Treatment; TiO_2; SLA Surface; Sandblasted/Acid-Etched Implant Surfaces; Implant Surface; Bone Implant Contact; Hydrophilic Surface*

Introduction

The tissue response to biomaterials is influenced by nano, micro and macrotopography of their surface [1]. It is well established that characteristics of implant surfaces, such as nano and micro-topography, and physicochemical composition, have a major influence on the outcome of osseointegration, especially at the histological level, aiming at biological and morphological compatibilities [2].

Most probably there is an optimal microroughness that affects the initial healing processes [3]. The optimal surface roughness has not yet been determined, even if Han., *et al.* have reported that a surface roughness of 1.5 μm produced a stronger bone response than smoother or rougher surfaces [4]. Current efforts to shorten the healing times of lifelong dental implants have focused on using surface-modification treatments.

Titanium's ability to withstand the harsh bodily environment is a result of the protective oxide film that forms naturally in the presence of oxygen. The oxide film is strongly adherent, insoluble, and chemically impermeable, preventing reactions between the metal and the surrounding environment. The native oxide that forms on titanium surfaces upon exposure to air is TiO_2, but lower oxidation states such as Ti_2O_3 and TiO have also been observed to exist in ambient conditions [5,6]. To achieve faster osseointegration, the surface of implant Titanium samples was subjected to thermal treatment. The aim of this preliminary study is to examine the influence of thermal treatment of Titanium dental implants on bone healing in rabbits.

Materials and Methods

Surface treatment

Threaded commercial titanium alloy implants (4 mm diameter and 13 mm length) were used in the present study. The textured Ti surfaces were obtained through acid-etching of plateau root form endosseous Titanium bulk alloy implants of 4 mm in diameter by 13 mm in length. The oxidation treatment was performed at 820°C for 1 minute in air. The test implants were placed at the center of the furnace and connected to a temperature controller to maintain a working temperatures of 802°C. After oxidation, samples were removed from the furnace and cooled in distilled water. The experimental set of implants was treated immediately prior to implantation while the control implants were left untreated.

Implant Placement

The protocol was approved by the Ethical Committee of University of Chieti-Pescara, Italy. Two skeletally pathogen free (SPF) and virus antibody free (VAF) mature male New Zealand white rabbits (Crl:KBL(NZW) of 9 months old and 3.5 Kg weight, obtained from Charles River Laboratories (Lieu-dit Oncins, France) were used in this study. The implants were inserted into the femoral knee. Each rabbit received two implants, one implant in the right femur and one in the left femur. A total of 4 implants (2 test and 2 test) were inserted. 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, 1 ml of 2% lidocain/adrenalin solution, was applied. A skin incision with a periosteal flap was used to expose the bone surface. The osteotomies were prepared with a 2-mm pilot bur used on a specially designed electric machine operated at 1,100 rpm with saline irrigation. The subsequent drilling was completed with slow speed sequential drilling with burs of growing diameter (2.5 to 4.5 mm) used on a handpiece operated at 50 rpm without saline irrigation. The periosteum and fascia were sutured with polyglycolic acid and the skin with silk (Sweden e Martina, Italy). Oxytetracycline dihydrate (Terramicina long Acting by Pfizer Italia srl) 100 mg/kg single dose and analgesics with tramadol hydrochloride (Altadol Abiogen Pharma S.p.A Italia) were given for 1 week. Sutures were removed 2 weeks after surgery. Postsurgical visits were scheduled daily to check the course of healing. No complications or deaths occurred in the postoperative period. All animals were euthanized with an overdose of intravenous pentobarbital at 8 weeks. A total of 4 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 0.15M cacodylate buffer at 4C 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). They were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany Leitz, Wetzlar, 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 3 slides were obtained for each implant. The slides were stained with acid fuchsin and toluidine blue. They were observed in normal transmitted light under a Leitz Laborlux microscope (Laborlux S, Leitz, Wetzlar, Germany). Histomorphometry of bone-implant contact percentage was carried out using a light microscope 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 and Computer Snc Milano, Italy).

Two implants for each group were analyzed under a Leo scanning electron microscope (Zeiss, Hallbergmoos, Germany).

Statistical evaluation

Differences between the groups of treatment were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) post-hoc test. A p-value < 0.05 was considered statistically significant. Data treatment and statistical analysis were done by Excel, Origin and SPSS software.

Results

Light Microscopy

Control implant

Newly-formed, strongly stained, bone was found in close contact with the implant surface (Figure 1A). The bone trabeculae were wide and contained large osteocyte lacunae. The osteoblasts were actively secreting the osteoid matrix that, in some areas, was undergoing mineralization.

An increase in the quantity of bone around the implants was observed. Only a few osteoblasts were present. Mature mineralized bone and, only in a few areas, not yet mineralized osteoid matrix were detected at the interface (Figure 1C). The bone-implant contact percentages was 54 ± 5%.

Test implant

It was possible to observe a large number of newly-formed, intensely stained, bone trabeculae that were in contact with the implant surface (Figure 1B). A few osteoblasts were actively secreting the osteoid matrix that, in some areas, was undergoing mineralization (Figure 1C). The absence of necrotic processes of the pre-existing bone when in contact with the implants was clearly observed. The bone-implant contact percentages was 68.83 ± 6.8%.

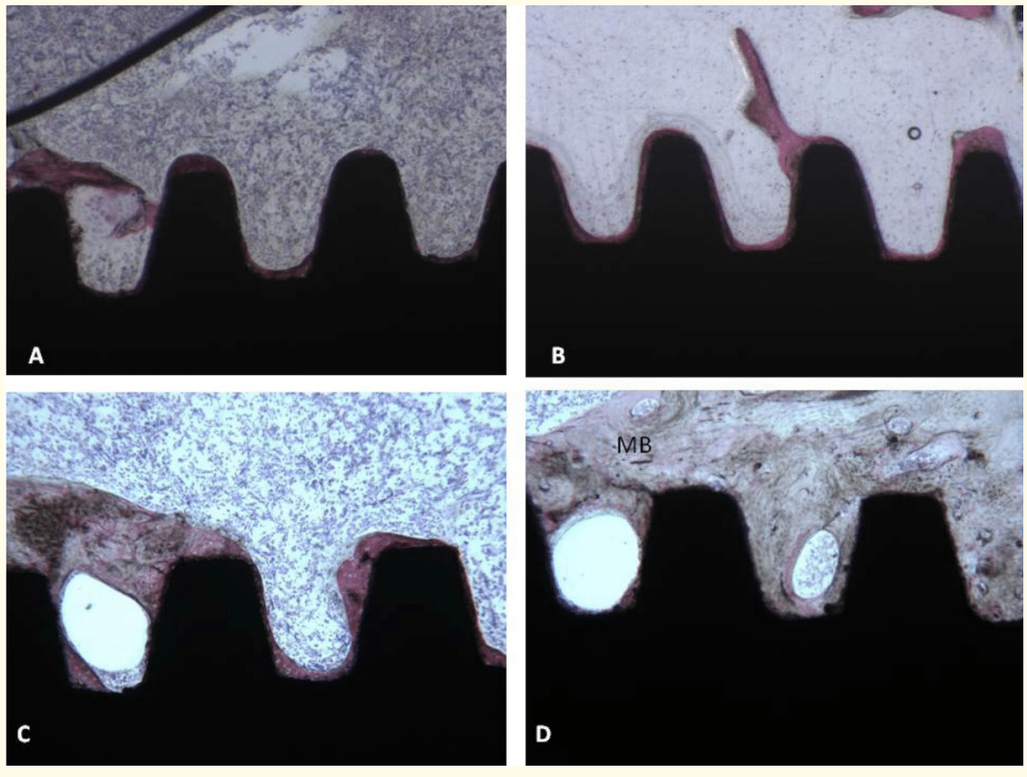

Figure 1: *Control (A) and test (B) implant after 8 weeks of healing. Higher bone formation was observed inside the threads of test implants (B) compared with control.*

Toluidine blue and acid fuchsin 12X

(C) At higher magnification, mature bone was present around the control implant. Toluidine blue and acid fuchsin 20X. (D)- At higher magnification, more mature bone trabeculae were present around the test implant surface (MB). Toluidine blue and acid fuchsin 20X.

Statistical analysis

The histological results showed bone-implant contact percentages on both implant surfaces. No statistically significant differences were found in the percentages of bone that had formed in the test group than in the control group implants in the specimens retrieved after 8 weeks ($p = 0.156$). In this study no statistical difference was found due to the insufficient number of specimens.

Discussion

In the present study it was found that the bone formation started preferentially in the implant modified by thermal treatment. In fact, although no significant difference has been detected, a higher difference was found in the percentages of bone implant contact that had formed on the implants modified by thermal treatment than on the control implants after 8 weeks. The BIC measurement of osseointegrated implants is the standard for the evaluation of bone formation on an implant surface. High BIC values are considered to be a prerequisite for implant stability, which clinically enables functional dental reconstruction. Current efforts to shorten the healing times of lifelong dental implants have focused on using surface-modification treatments. The approaches which have been found to be beneficial to the biological performance of the implants include increasing the surface roughness [7], as well as oxidation to form thicker or otherwise modified TiO_2 layers on the surface [8]. This study proved the increase of BIC effect on implants with the surface modified by thermal treatment as there was a marked bone neoformation with active mineralization processes on the implant surfaces.

Different methods have been used to increase the TiO_2 layer, thermal, anodix [9] anodic oxidation [10], heat, enhancing osseointegration and improving initial stability [11-13]. A previous study has demonstrated that TiO_2 on the transmucosal portion of the abutment reduces the quantity of bacteria that attaches to the metal surface and produces more healthy peri-implant tissues [14]. Also, a coating of the implant body could be hypothesized, with positive effects in cases of peri-implant crestal bone resorption during peri-implantitis, when a TiO_2 that decreases the bacterial charge could be helpful in the treatment of peri-implant infection.

In this experimental study, all the implants were similar with regard to dimension, chemical surface composition, but differed in terms of surface oxide thickness, surface topography and crystal structure. It was demonstrated that the increase in roughness at microscale of specimens due to the thermal treatment at 700°C for 1h was not significant [15]. *In vitro* experiments with primary osteoblast cell culture revealed that the surface modification does not alter but may improve the excellent biocompatible behaviour. In fact, cell adhesion is favoured on the thermally treated surfaces [16].

In conclusion these preliminary results suggest that surfaces modified by thermal treatment promote bone response in direct contact with the implant surface and could be clinically advantageous for shortening the implant healing period, for implants placed in areas with low density bone. The outcome of this preliminary study in rabbit allows us to set up and conduct a study with a higher sample number and supports the need to perform a study with a statistical value.

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