

Analysis of the Relation between Different Titanium Surfaces and Bone-Implant Contact

Antonio Carlos Aloise¹, Luís Guilherme Scavone de Macedo¹, Rafael de Mello e Oliveira¹, Jaime Sardá Aramburu Júnior², Marcelo Sperandio³, Marcelo Henrique Napimoga³, Marcelo Lucchesi Teixeira⁴, André Antonio Pelegrine⁴

¹Implant dentistry and prosthodontics Sao Leopoldo Mandic Dental School and Research Institute, Campinas SP Brazil

²Surgery and anesthetics in small animals Faculty of Itapiranga, Itapiranga/SC Brazil

³Histology and pathology Sao Leopoldo Mandic Dental School and Research Institute, Campinas SP Brazil

⁴Implant dentistry and oral rehabilitation Sao Leopoldo Mandic Dental School and Research Institute, Campinas SP Brazil

Original Research Article

*Corresponding author
André Antonio Pelegrine

Article History

Received: 18.10.2017

Accepted: 27.10.2017

Published: 30.10.2017

DOI:

10.21276/sjds.2017.4.10.9



Abstract: The aim was to evaluate bone-implant contact (BIC) of implants from different brands and different surface treatments. 24 implants were divided into four groups: Group IO Intraosseous implants with doubly acid-etched surface, NEO group Neodent implants with sandblasted and acid-etched surface, NB group Nobel Biocare implants with anodized surface and TF group TitaniumFix implants with sandblasted and etched surface. Six adult male New Zealand rabbits received two implants in each tibia. After 45 days of implant placement all animals were sacrificed. The bone blocks removed were analyzed histomorphometrically for quantification of the bone-implant contact area. Qualitative analysis was performed on the topographical aspects of implant surface from groups IO, NEO, NB and TF through using scanning electron microscopy (SEM). The BIC percentage for groups IO, NEO, NB and TF were $53.8 \pm 11.2\%$, $59.5 \pm 14.3\%$, $61.2 \pm 2.9\%$ and $42.8 \pm 2.9\%$, respectively. Qualitative analysis of SEM images showed that the implant surfaces from the four groups had different topographical features, which conformed to their respective surface treatment methods. NB group showed a higher percentage of BIC area, followed by NEO, IO and TF, respectively. Qualitative analysis of topographic morphology by SEM showed characteristics compatible with increased surface roughness.

Keywords: dental implants, osseointegration, bone-implant contact, histomorphometry

INTRODUCTION

Osseointegration was defined as the direct structural and functional connection between living bone and implant surface under load [1]. The importance of creating roughness on the implant surface to enhance osseointegration was suggested by Andrew Schroeder [2]. Various surface modification techniques have since been tested, either by addition methods, such as plasma titanium spray [2], plasma spraying hydroxyapatite [3] and anodizing [4] or subtraction methods, such as sandblasting [5], etching [6], sandblasting followed by etching [7] and laser [8]. The creation of hydrophilic surfaces is among the most recent advances under investigation in this subject [9].

There is consensus in the scientific literature that bone-implant contact (BIC) is not uniform. The quality of osseointegration depends on the percentage of direct contact between bone and implant, with surface characteristics being an important tool for improving of quality, especially in low-density bone tissue [10]. Preclinical trials have shown that surface treatments not only increase implant removal torque when compared to machined surfaces show, but also BIC [11,12]. Changes to implant surface also reduce the time needed for osseointegration [13,14], which in turn make the restorative process more efficient.

Despite recent advances in the incorporation of biomolecules to implant to enhance osteoblast adhesion, proliferation and differentiation [15,16] the actual longevity of the active surface and the costs involved with such technology still prevent its routine use. Consequently, cost-effectiveness dictates which surface treatments will be most commonly used by the implant industry, with subtraction methods being the most popular, especially etching and sandblasting plus etching. The aim of this study was therefore to evaluate the bone-implant contact of commercially pure titanium

implants from different brands and different surface treatments.

MATERIALS AND METHODS

Twenty-four internal connection dental implants measuring 3.5 mm in diameter and 8.0 mm in length were divided into four groups according to manufacturer. They were group IO (n = 6), Intraoss implants (Titaoss, Intraoss, Itaquaquecetuba, SP, Brazil) with surface treated by double acid etching; group NEO (n = 6), Neodent implants (Titamax Cortical, Neodent, Curitiba, Brazil) with surface treated by sandblasting followed by etching, group NB (n = 6), Nobel Biocare implants (Replace Select, Nobel Biocare, Danaher Corporation, Washington, DC, USA) with surface treated by anodizing and group TF (n = 6), Titanium Fix implants (Black Fix, Titanium Fix, São José dos Campos, SP, Brazil) with surface treated by blasting followed by acid etching.

Surgical technique

This study was approved by the ethics committee on animal research of the Faculty of Itapiranga (Itapiranga, SC, Brazil) under the registration number 004-09-2015. Six male adult New Zealand rabbits weighing between 3.5Kg and 4.0kg received an implant from each group in their left and right tibias.

All animals were treated under general anesthesia via an initial intramuscular injection of ketamine (35 mg/kg; Agener Pharmaceutica, Brazil), followed by a muscle relaxant Rompum (5 mg / kg, Bayer, Brazil) and a tranquilizer Acepran (0.75 mg/kg Univet, Brazil).

Immediately prior to surgery, the rabbits had their fur shaved around the tibias to be implanted using an electrical appliance and the skin was decontaminated with 0.2% chlorhexidine (Rioquímica, São José do Rio Preto, SP, Brazil). Additionally, 1 ml of local anesthetic (3% Prilocaine-Felipressine, Astra, Mexico) was injected subcutaneously into the surgical site to enhance analgesia and control bleeding. An incision of approximately 3.0 cm with subsequent flap raising and periosteum detachment was performed on each tibia. Two implants were then placed in the proximal metaphysis of the tibia and two in the distal portion, totalizing four implants per animal. Each implant received its respective cover screw. The soft tissue was sutured in layers using nylon sutures (Ethicon® 5.0; Johnson & Johnson Medical Ltd., Blue Ash, Ohio).

Bone drilling for implant placement was performed sequentially following the instructions by the manufacturers using the specific set of drills for each implant. A rotating device was used for osteotomy, which was set at 1.000 rpm under saline irrigation. The implants were installed manually at the bone level aided by a ratchet, so that the first thread was fully below the bone level. After surgery, the animals were placed in individual cages, where they received a single dose of Benzetacil 600,000 IU. They were maintained under 12-hour cycles of light, temperature controlled at 21 °C and diet *ad libitum*.

After 45 days of implant placement, all animals were sacrificed with an intravenous injection of Ketamine (2 ml) and xylazine (1 ml). The anatomical pieces under investigation were removed using diamond disks under irrigation and immediately taken to the histology laboratory for processing.

Histological Procedures

The bone biopsies containing the implants were initially processed by fixation in 10% formalin for 48 hours, rinsed under running water for 12 hours and gradually dehydrated by immersion in a sequence of ethanol solutions (60%, 70%, 80%, 99%) for 24 to 56 hours. After dehydration, they were embedded in Technovit 7200 VLC resin (Kulzer & Co, Wehrheim, Germany). Two slices from each specimen were then cut longitudinally on a precision cutter using a diamond disc. The sections were stained with hematoxylin-eosin and bone neoformation was evaluated on the surface of the implants. Light microscope images were obtained (Nikon E200) at 40x and 400x magnifications (Fig. 1) to assess BIC and the characteristics of the newly formed bone.

Histomorphometric analysis

All histological sections were identified by a random sequence of numbers in order to encode the samples for an independent examiner. The linear measurements of the BIC area obtained were evaluated on Image Tool version 5.02 for Microsoft Windows from microscopic images at 400x magnification. The percentage of direct contact between bone and implant fully inserted into bone was considered for calculation.

Scanning Electron Microscopy (SEM)

An implant from each brand was evaluated under scanning electron microscopy (SEM) at 100, 500, 1000 and 5000 times magnifications. A Philips XL30 microscope was used to obtain the images in BSES mode (backscattered electrons) (Figs. 2-5).

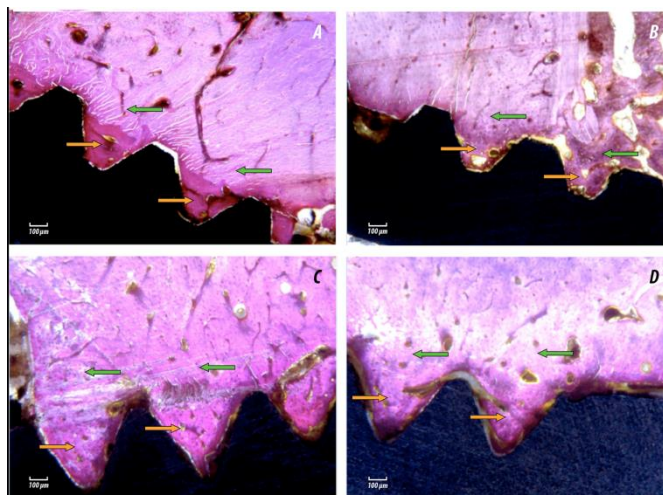


Fig-1: Photomicrograph of the implants and surrounding bone. HE stains 40X magnification. Orange arrows: Newly formed bone; Green arrows: Lamellar bone. a-(Titamax Cortical); b-(Titaoss); c-(Replace Select); d-(Black Fix).

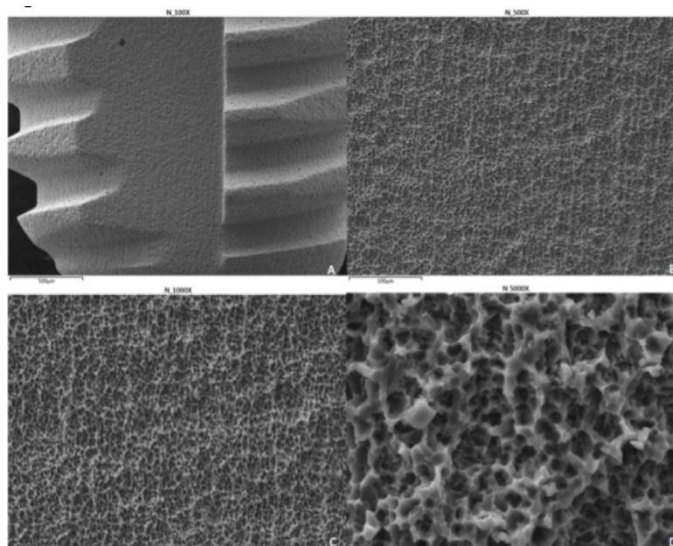


Fig-2: Photomicrographs of the Titamax Cortical implant surface obtained by scanning electron microscopy (SEM) at magnifications: (A) 100X; (B) 500X; (C) 1000X, and (D) 5.000X.

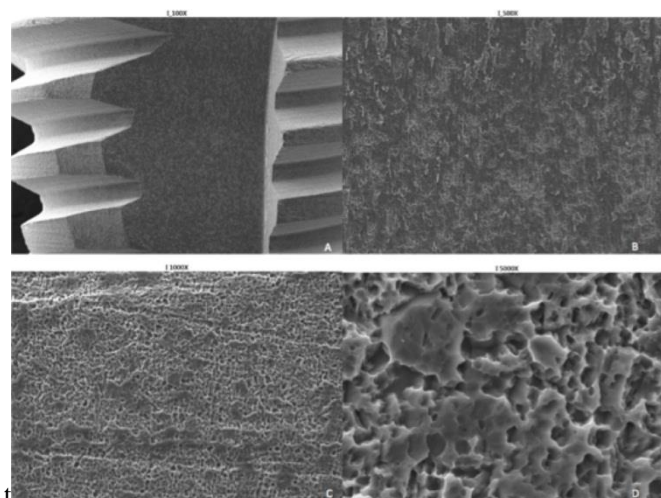


Fig-3: Photomicrographs of the surface of the implant Titaoss obtained by scanning electron microscopy (SEM) at the following magnifications: (A) 100X; (B) 500X; (C) 1000X, and (D) 5.000X.

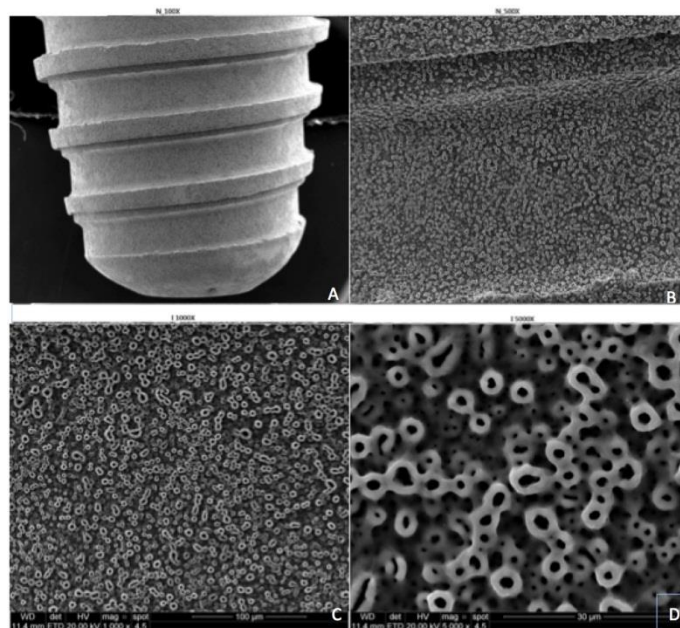


Fig-4: Photomicrographs of the Replace Select implant surface obtained by scanning electron microscopy (SEM) at the following magnifications: (A) 100X; (B) 500X; (C) 1000X, and (D) 5.000X.

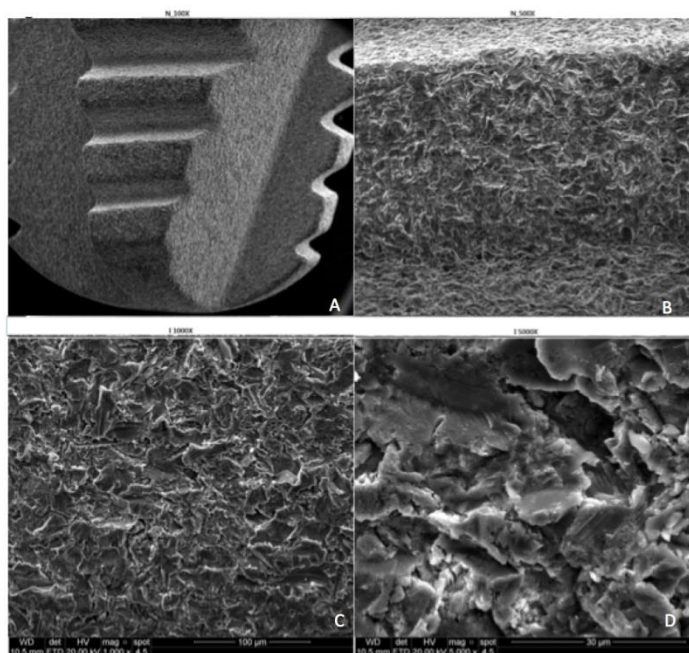


Fig-5: Photomicrographs of the Black Fix implant surface obtained by scanning electron microscopy (SEM) at the following magnifications: (A) 100X; (B) 500X; (C) 1000X, and (D) 5.000X.

Statistical Analysis

The nonparametric Kruskal-Wallis test was used for overall comparative analysis of the histomorphometric data and the nonparametric pairwise Wilcoxon test was used to establish the difference

detected by the previous test, using a significance level of 0.05.

RESULTS

Histological observations

Table-1 : Analysis of the Bone-Implant Contact Surface (%)

Rabbits	IO	NEO	NB	TF	P value
1	55	60	63	41	
2	58	58	66	45	
3	57	62	59	47	
4	48	61	61	43	
5	53	57	58	39	
6	52	59	60	42	
Mean	53.8	59.5	61.2	42.8	
SD	11.2	14.3	17.7	12.5	
Median	53.7	59.7	62.1	42.2	0.04*

The qualitative assessment of the samples from the four groups showed new bone formation at the implant surface, as observed from the hematoxylin and eosin sections. The new bone formed had the characteristics of immature bone compared to the adjacent hard tissue, which presented a lamellar pattern. Data for quantitative analysis of BIC and intergroup comparisons are found in tables 1 and 2, respectively.

Values expressed in percentage of bone-implant contact (BIC) area obtained by histomorphometry. Group IO: doubly acid-etched implant surface by IntraOsss (n = 6); Group NEO: sandblasted and acid-etched implant surface by Neodent (n = 6); Group NB: anodized implant surface by Nobel Biocare (n = 6) and Group TF: sandblasted and acid-etched implant surface by Titanium Fix (n = 6). Kruskal-Wallis test, with p values <0.05 considered significant *

Table-2 : Intergroup comparisons

	P value
IO x NEO	0,03*
IO x NB	0,04*
IO x TF	0,03*
NEO x NB	0,2
NEO x TF	0,03*
NB x TF	0,02*

Group IO: doubly acid-etched implant surface by IntraOsss (n = 6); Group NEO: sandblasted and acid-etched implant surface by Neodent (n = 6); Group NB: anodized implant surface by Nobel Biocare (n = 6) and Group TF: sandblasted and acid-etched implant surface by Titanium Fix (n = 6). Wilcoxon test, with p values <0.05 considered significant *.

Topographic analysis of implant surface

Topographic evaluation of the implant surfaces was performed on the four groups using scanning electron microscopy of means (SEM). Qualitative analysis of the images illustrated distinct implant surfaces, which were compatible with the respective surface treatment methods of the four study groups.

DISCUSSION

Three types of commercially pure grade IV titanium dental implants available in the Brazilian market as well as a reference implant in the global market, all treated with different micro-texturization methods, were placed in the tibia of rabbits to assess osseointegration. The surface treated by double etching was represented by the Titaoss implants (Intraoss, Itaquaquecetuba, SP, Brazil), group IO. Surfaces treated with sandblasting followed by etching were represented

by both the Titamax Cortical implants (Neodent, Curitiba, Brazil), Group NEO, and the Titanium-fix implants (Black Fix, Titanium-fix, São José dos Campos, SP, Brazil), Group TF. The anodized surface was represented by the Nobel Biocare implants (Replace Select, Nobel Biocare, Danaher Corporation, Washington, DC, USA) Group NB.

Three topographical features are considered important in the development of a metal surface: chemical aspects, surface charge and wettability [17]. Reports have shown that biological aspects involving the functionality of metallic devices can be affected by the properties of the metal surface, namely protein adsorption capacity, cell-surface interaction and the quantity and quality of the biological tissue at the interface between the biological environment and the metal surface [18,19].

Metal oxidation is responsible for the passivation of the surface, which occurs by mechanisms of hydroxylation and hydration, where titanium oxide plays such a role in the case of titanium implants. This process is directly related to surface energy and influences the degree of contact between the implant surface and the biological environment [20,21].

Classification of implants is generally based on the type of biomaterial used to manufacture them, for instance titanium, titanium alloys, ceramics, polymers and composites. The surface of such implants can also be classified according to the type of surface treatment: machined, macro-textured, micro-textured and biomimetic nano-textured [14].

Micro-textured surfaces can be obtained by subtraction, e.g. acid etching, which produces surfaces with an average roughness (Ra) of 1.30µm. Ciotti [22] used implant surfaces treated by double etching, which induced micromorphologic changes to the implant surface that increased the contact area between the mineralized bone and the implant. Such modification caused the implant surface to become rough, thus increasing torque removal and favored bone deposition.

Acid etching can be performed after sandblasting with large particles of aluminum oxide (250 - 500µm) using sulfuric acid or hydrochloric acid. This is known as the SLA surface (S = sandblasted; L = large grit; A = acid etching). This type of surface combines macro-texturization by sandblasting with micro-texturization by acid etching [14]. Surfaces treated by sandblasting and etching have also shown to favor biocompatibility and bone neof ormation around the implant. The topographical outcome of such surface treatment includes the creation of small uniform craters (1 to 2 micrometers in diameter) and peaks [23], which can also be seen on surfaces that have been anodized [4].

In this study, bone biopsies containing implants were removed on a single occasion after 45 days of implant placement. This was longer than the time reported by Beutel [24], who demonstrated a significant increase ($p < 0.001$) in bone-implant contact between 21 and 35 days. Their findings aided in designing the present study and in preventing the need for a further group, which in turn optimized the number of animals for experimentation.

The histomorphometric analysis showed the percentage of BIC, where a significant difference was observed between the groups IO, NEO, NB and TF, with mean values \pm standard deviation of $53.8 \pm 11.2\%$, $59.5 \pm 14.3\%$, $61.2 \pm 2.9\%$ and $42.8 \pm 2.9\%$, respectively. Despite the significant difference, these findings demonstrate that bone-implant contact level in all groups was higher than those reported by Lee [25], who evaluated the same animal model and BIC using surfaces treated by sandblasting plus etching ($15 \pm 11.4\%$) and machined surfaces as control ($11.2 \pm 7.8\%$). It is important to highlight that the type of bone (cortical or cancellous) as well as the topography of the rabbit tibia may influence the results. The studies by Beutel [24] demonstrated that BIC was higher ($p < 0.001$) in cortical bone when compared to cancellous bone.

Qualitative analysis of the topographical morphology between the groups demonstrated that in all surface treatment methods evaluated in this study the original metal surface was a modified, forming micro-retentions that, according to Kim [23], promote cell adhesion and migration.

CONCLUSION

The groups in which Nobel Biocare and Neodent implants were placed had a higher proportion of BIC area followed by the implants by Intraoss and TitaniumFix. Qualitative analysis of topographic morphology by SEM demonstrated that the four groups showed surface changes that translated into increased roughness.

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