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Osseointegration of chemically modified sandblasted and acid-etched titanium implant surface in diabetic rats: a histological and scanning electron microscopy study

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Abstract

Introduction: The aim of this study is to assess the osseointegration of different dental implants surfaces in diabetic rats. *Materials and Methods*: In this study, were used 56 male Wistar rats, average weight of 300–350 g. Diabetes was induced by a single intraperitoneal injection of Streptozotocin. The glucose levels and weight of rats were periodically evaluated. After the diabetes mellitus is confirmed, the sandblasted, large-grit, acid-etched (SLA) and SLActive endosseous dental implants (TAG dental implants, TAG Medical, Israel), made of titanium alloy, Ti-6Al-4V, 1 mm diameter and 3 mm in length were inserted in the distal metaphysis of the left femur. *Results*: Diabetic rats have naturally lower number of bone cells and bone-implant contact (BIC%) than healthy rats when using the SLA implant, but when using SLActive implant, diabetic and healthy rats have the same numbers. *Conclusions*: The use of the SLActive surface resulted in positive effects in healthy and especially in diabetic animals, which demonstrate that could improve the osseointegration progress in humans with diabetes.

Keywords: dental implants, osseointegration, diabetes mellitus, scanning electron microscopy, histology, histomorphometry.

Introduction

Oral implants, generally made of pure titanium or titanium-based alloys, are widely used in the prosthetic rehabilitation of fully and partially edentulous patients.

The ultimate goal in implant therapy is to achieve an early and strong implant fixation into the native surrounding bone tissue. Although titanium is commonly used as a favorable bone implant material due to its mechanical properties, its bioactive and osteoconductive capacity is relatively low [1]. Therefore, implant surface modification experiments intend to improve the early process of osseointegration, as characterized by an increased bone-to-implant contact and enhanced bone volume in the area surrounding the implant [2]. For this purpose, different surface modification approaches have been explored to optimize the interaction between implants and native bone tissue. By altering either surface topography (*i.e.*, grit blasting and acid etching) or changing the physicochemical properties of the surface (*i.e.*, coating deposition), both the bioactive and osteoconductive properties of the surface can be improved [3].

In view of topographical approaches, it is generally accepted that moderately roughened titanium implants have a superior influence on the bone response in comparison with polished "smooth" implant surfaces [3]. Alternatively, physicochemical surface alterations, such as coating deposition with osteopromotive compounds, have been shown to be of special interest in the contemporary field of research [4, 5].

In many cases, studies of osseointegration of implants in diabetic animals have involved a problem with study design: the time between the induction of diabetes and the implantation was not appropriate. Consequently, pathological changes in soft tissue or bone metabolism are not likely to have occurred. A study evaluating the establishment of a diabetic pig model showed that significant changes in blood vessels or hard tissues take place after 6–12 months at the earliest [6].

This study aims to assess by scanning electron microscopy (SEM) and histomorphometry, the osseointegration of sandblasted, large-grit, acid-etched (SLA) and SLActive implants surfaces and peri-implant bone in diabetic rats.

Materials and Methods

The implants used were TAG® dental implants (TAG Medical, Israel) made of titanium alloy (Ti-6Al-4V ELI) with a diameter of 1 mm and a length of 3 mm. Two surface modifications were tested. The TAG surface was used as a control group. This surface is sandblasted and acid-etched, resulting in a microroughened surface topology.

The experimental surface used was the surface

produced by conditioning the conventional sandblasted and acid-etched surface in a nitrogen atmosphere and preserving it in an isotonic sodium chloride solution to avoid contamination with extrinsic molecules (SLActive), which are otherwise deposited in the micropores of the surface.

Animal model and implant procedure

For this study, 56 male Wistar rats were acclimatized to the study conditions for a period of 14 days before the induction of diabetes. All experimental designs and procedures have received approval of the Animal Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania. The animals were housed individually, at 25^0C. They were strictly pair-fed a laboratory diet containing 15% casein, 0.8% phosphorus, 1% calcium, 70–80% carbohydrates, and 5% fat throughout the experimental period. Demineralized water was available *ad libitum*.

Diabetes has been obtained by intraperitoneally administration of Streptozotocin (Sigma-Aldrich, Dorset, UK) 40 mg/kg dissolved in 10 mM sodium citrate (pH 4.5), following sedation of the animals with Azaperone [1 mg/kg body weight (b.w.)] and Midazolam (1 mg/kg b.w.). The subjects were diagnosed with diabetes, if the values of glycemia were over 200 mg/dL. In this study, we evaluated the blood glucose levels measured by Accu-Check Active® glucometer.

At three months after the administration of Streptozotocin, the implants were placed in the left femur, according to protocol. All animals received peri-operative antibiotics (Penicillin G®, Jenapharm GmbH & Co. KG, Jena, Germany) from one hour before surgery to five days after surgery, to reduce the risk of infections. Anesthesia was initiated with Ketamine HCl (Ketavet®, Ratiopharm, Ulm, Germany), followed by administration of inhalation anesthesia (Isoflurane®, Pharmacia & Upjohn GmbH, Erlangen, Germany). A local anesthetic (Ultracain® D-S Forte, Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany) was also injected into the left femur of each animal, an incision was made at the distal metaphysis of the left femur and the bone was uncovered.

After the randomized placement of two implants per animal (each one SLA® and one SLActive® implants per animal; 112 implants in total), according to the standard protocol for the implant system used (TAG® implants, TAG Medical, Israel), the periosteum and skin were sutured in two layers (Vicryl 5-0, Ethicon GmbH & Co. KG, Norderstedt, Germany) (Figure 1). To reduce post-operative pain, Butorphanol (0.05 mg/kg b.w.) was injected subcutaneously every 12 hours for five days.
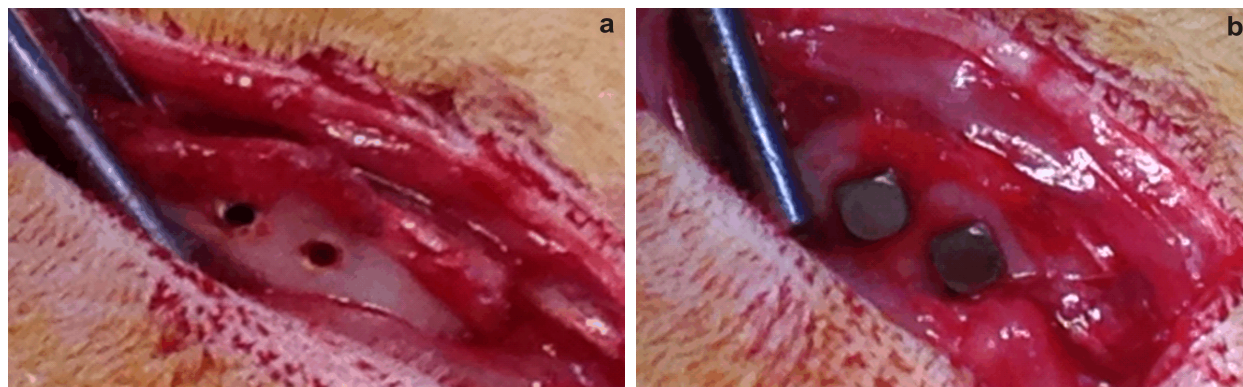
Figure 1 – *(a) Preparation of osteotomies. (b) The SLA and SLActive implants inserted. SLA: Sandblasted, large-grit, acid-etched.*

At 60 days after surgery, 10 healthy and 10 diabetic animals were sacrificed, representing an early implant loading approach. Two diabetic animals died before day 60 and were therefore excluded from the study. The remaining 18 healthy and 16 diabetic animals were sacrificed at 120 days after surgery.

Each animal was sedated with an intramuscular injection of Ketamine (40 mg/kg b.w) and Midazolam (1 mg/kg b.w), followed by an intravascular injection of 20% Pentobarbital solution into an ear vein until cardiac arrest occurred. The finding of absence of vital signs (respiratory movements, heartbeat, reflexes) animals will be dissected to harvest the femur.

SEM analyses

Immediately after euthanasia of animals, all samples were provided by performing an incomplete osteotomy with a rotary cylinder at the site where each implant has been inserted, and subsequent fracture of the bone fragments in the area of weakness. Subsequently, the samples were prefixed with 3% Glutaraldehyde solution and stored at 40^0C until the examination by electron microscopy. After obtaining the biological samples, they were examined by SEM using a microscope type Tescan Vega II LMU with an accelerating voltage of 30 kV electron beam, scoring a 30 μA current sample, a diameter of electron beam interaction with the sample surface corresponding to a distance between the spot four, one pole piece of the microscope and the surface of the sample between 10 and 13 mm.

One quantitative parameter was assessed:

▪ **Number of bone cells (NrO)**. The number of the bone cells from the surface of the implants was assessed in four rectangular regions of interest (ROIs); ROI 1 and ROI 2 were defined from the first coronal up to the third microthread of the implant; ROI 3 and ROI 4 were defined for the last three microthreads of the apical region of the implant. Number of bone cells was defined as the total number of bone cells found between the microthread from each region of interest (Figure 2). The bone cells are represented by the all bone cells: osteoblasts and osteocytes attached to microthreads of the implant.

Histological and histomorphometry analyses

The bone samples were fixed in 10% neutral buffered formalin (24–48 hours) and then decalcified in Bouin's

solution 72%. After tissue processing, the specimens were embedded into paraffin blocks (Leica TP1020, Leica Microsystems GmbH, Germany). The paraffin blocks were cut into 5 μm sections using Microtome SLEE CUT 6062 (SLEE Medical GmbH, Germany). The sliced sections were deparaffinized and stained with Masson's trichrome techniques.

Histomorphometry was performed using digital image analysis software (Leica Qwin Pro-image Leica Imaging Systems, Cambridge, UK).

One quantitative parameter was assessed:

▪ **Percentage of bone-to-implant contact (BIC%)**. Bone contact was assessed along the total length of the implant in four rectangular regions of interest, starting at the first coronal up to the third microthread of the implant for ROI 1 and ROI 2 and for the last three microthread of the apex of the implant for ROI 3 and ROI 4. BIC% was defined as the percentage of the implant surface in direct contact with bone without intervening fibrous tissue layers (Figure 3). BIC% was calculated as total BIC divided by total circumference implant from each region of interest ×100.
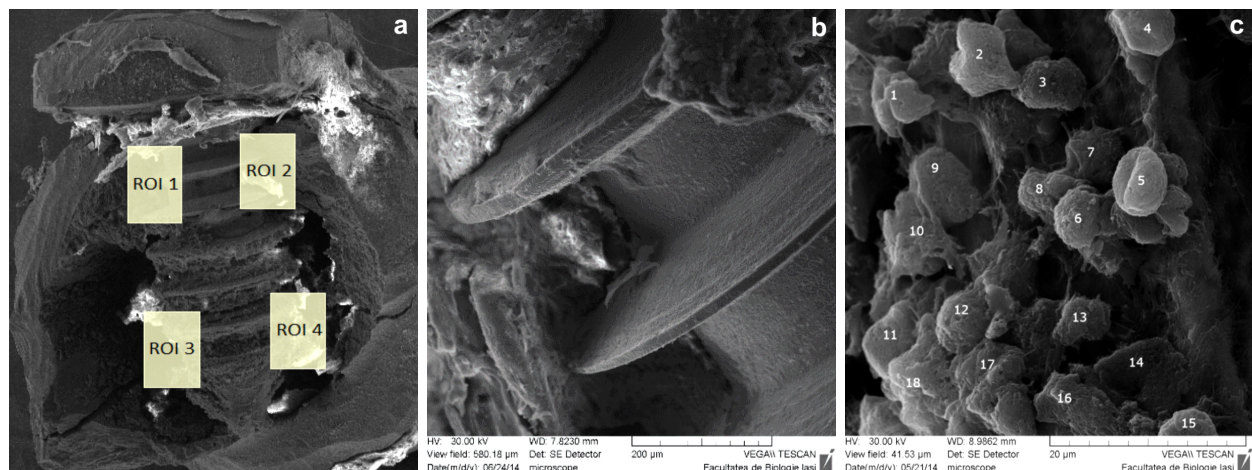

Figure 2 – *Four regions of interest (ROIs) for the evaluation of the number of the bone cells in the SLA and SLActive implants groups. SLA: Sandblasted, large-grit, acid-etched.*
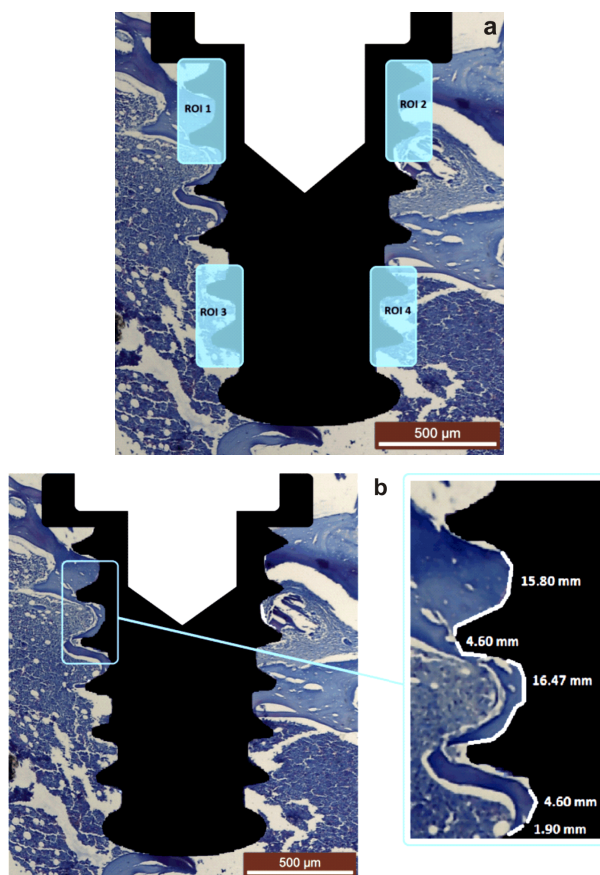

Figure 3 – *Four regions of interest (ROIs) for the evaluation of bone implant contact in the SLA (a) and SLActive (b) implants groups. SLA: Sandblasted, large-grit, acid-etched.*

Statistics

All measurements were statistically evaluated using Statistical Package for the Social Sciences (SPSS) 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Mean values and standard deviations (SDs) were calculated. Mann–Whitney test for non-parametric statistics was performed for the analysis of BIC and number of bone cells. A Wilcoxon signed-rank test was performed, with a significance level set at $p<0.005$.

⊟ Results

Clinical results

At 60 days after surgery, when the sacrification was performed, it was clinically found out that two implants with sandblasted and acid-etched surface from each group, healthy and diabetic, were not osseointegrated. At 120 days after surgery, it was clinically found out that three implants with sandblasted and acid-etched surface and one implant with experimental surface from diabetic group were not osseointegrated.

Scanning electron microscopy results

In the healthy rats, there is a significant difference in the mean bone cells number between day 60 and day 120, when the SLA implant and when the SLActive implant are used, the number of bone cells being higher at day 120 compared to day 60. In the healthy rats, there is a significant difference in the mean bone cells number at day 60 and at day 120, between SLA implant and SLActive

implant, the number of bone cells being higher with the SLActive implant compared to the SLA implant.

In the diabetic rats, there is no difference in the bone cells number between day 60 and day 120, when the SLA implant and SLActive implant are used. In the diabetic rats, there is a significant difference in the number of bone cells between the SLA implant and the SLActive implant at day 60 and day 120.

Using the implant, the healthy rats have an increase in the number of bone cells between day 60 and day 120, and the diabetic rats do not have this increase. Using the SLA implant, there is no difference in the number of bone cells between diabetes and healthy rats at day 60, but there is a significant difference at day 120 (Table 1).

Table 1 – *Mean values of bone cells number*

	Day 60	**Day 120**
Implant SLA, diabetic	235.500 (43.3)	237.462 (31.6)
Implant SLA, healthy	248.125 (52.4)	304.167 (46.8)
Implant SLActive, diabetic	410.000 (57)	478.200 (91)
Implant SLActive, healthy	424.100 (53.8)	538.611 (70.5)

SLA: Sandblasted, large-grit, acid-etched.

Histological and histomorphometric results

In the healthy rats, there is a significant difference in the mean BIC% between day 60 and day 120, when the SLA implant and when the SLActive implant are used, the BIC% being higher at day 120 compared to day 60. In the healthy rats, there is a significant difference in the mean BIC% at day 60 and at day 120 between SLA implant and SLActive implant, the BIC% being higher with the SLActive implant compared to the SLA implant. SLActive implant increases the number of bone cells and BIC% at day 60 and day 120 in the healthy rats.

In the diabetic rats, there is a significant difference in the mean BIC% between day 60 and day 120, when the SLA implant and when the SLActive implant are used, the BIC% being higher at day 120 compared to day 60. In the diabetic rats, there is a significant difference in the mean BIC% at day 60 and at day 120, between SLA implant and SLActive implant, the BIC% being higher with the SLActive implant compared to the SLA implant.

In the diabetic rats, there is no increase in the number of bone cells between day 60 and day 120 but there is an increase in the BIC% (Figures 4 and 5). The number of bone cells and BIC% is higher with SLActive implant than with the SLA implant. SLActive implant increases number of bone cells and BIC% in healthy and diabetic rats at day 60 and at day 120. Using the implant, the healthy rats and the diabetic rats have an increase in BIC% between day 60 and day 120. There is a significant difference in the BIC% between diabetes and healthy rats at day 60 and at day 120.

Diabetic rats have naturally lower number of bone cells and BIC% than healthy rats when using the SLA implant, but when using SLActive implant, diabetic and healthy rats have the same numbers (Table 2).
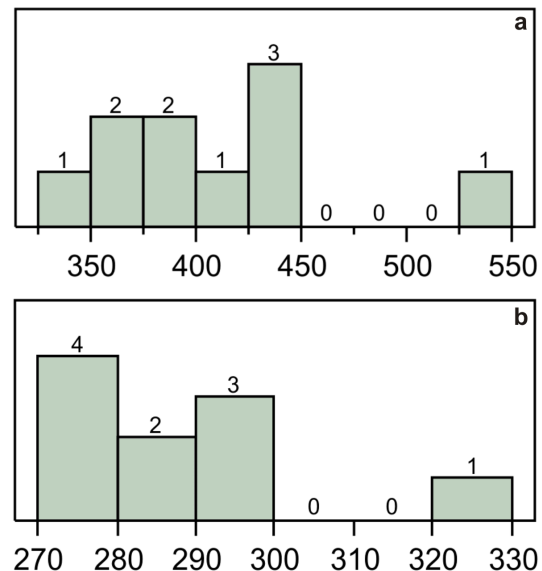
Figure 4 – *Distribution of number of bone cells (a) and BIC% (b) at 60 days after surgery at the diabetic subjects with experimental implant surface (SLActive). SLA: Sandblasted, large-grit, acid-etched.*
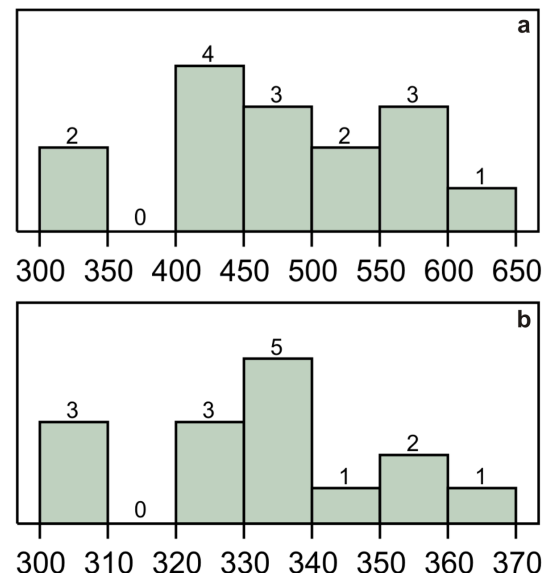
Figure 5 – *Distribution of number of bone cells (a) and BIC% (b) at 120 days after surgery at the diabetic subjects with experimental implant surface (SLActive). SLA: Sandblasted, large-grit, acid-etched.*

Table 2 – *Mean values of BIC%*

	Day 60	**Day 120**
Implant SLA, diabetic	150.125 (27.8)	182.077 (27)
Implant SLA, healthy	221.375 (43.2)	290.222 (45.5)
Implant SLActive, diabetic	287.700 (16.8)	331.533 (18.2)
Implant SLActive, healthy	309.400 (25.1)	335.889 (20)

BIC%: Percentage of bone-to-implant contact; SLA: Sandblasted, large-grit, acid-etched.

⮡ Discussion

The primary question of this study was the evaluation of the BIC in diabetic compared with healthy animals. Diabetes was not treated in this study, so the hard tissue around the implant was premature and less organized

compared with healthy animals, as seen in previous studies [6, 7]. A quantitative and qualitative deficiency of the peri-implant bone in diabetic compared with healthy organisms is therefore expected [8]. The TAG surface used as a control group is sandblasted and acid-etched, resulting in a microroughened surface topology. The outcome is improved adhesion of bone cells [9, 10].

The experimental surface used was a conditioning conventional sandblasted and acid-etched surface in a nitrogen atmosphere and preserving it in an isotonic sodium chloride solution to avoid contamination with extrinsic molecules (SLActive), which are otherwise deposited in the micropores of the surface. Thus, the surface becomes hydrophilic, creating an ideal wettability and ideal conditions for the apposition of blood and bone cells [11–16]. The current study showed that diabetes led to significantly lower BIC% rates after 60 and 120 days.

After 120 days, the BIC% of SLActive implants in diabetic animals was significantly higher than the contact value of the SLA implants. Furthermore, the value of the SLActive implants in diabetic animals reached the BIC% found in the SLA group in healthy animals. The modified surface thus compensated for the negative influence of diabetes mellitus on osseointegration. Although findings in the literature support these results of an improved osseointegration of SLActive implants, a positive effect of this surface on osseointegration in healthy animals was not confirmed in our study [11, 13].

Because of the modified surface design of the SLActive implants, this hydrophilic surface seemingly leads to improved differentiation of bone cells and enhanced expression of growth factors, like in chronic thyroiditis [17]. The histomorphometric results in diabetic animals can be explained by the pathological changes identified in previous studies, such as reduced bone cells' expression, reduced osteoid production, and impaired bone apposition to implants [18–24].

⊟ Conclusions

Diabetes mellitus has pathological effects on osseo-integration of dental implants. This study showed an impaired BIC% in the diabetic group at both points in time. The use of the hydrophilic SLActive surface resulted in positive effects in healthy and diabetic animals. Therefore, this surface seems especially suitable for compromised patients.

Conflict of interests

The authors declare that they have no conflict of interests.

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