Scanning Electron Microscopy Investigation of Bone Apposition Around Two Different Sandblasted Acid - etched Titanium Implant Surfaces in Diabetic Rats

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This aim of this study is to assess the stages of formation and maturation of peri - implant bone trying to make a correlation between the ability of osseointegration of different dental implants surfaces in diabetic rats. In this study, were used 60 Wistar rats, male, average weight from 400 - 450 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, 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.

Keywords: dental implants; osseointegration: diabetes mellitus, SEM

Dental implants are an excellent treatment option for restoration of the missing teeth. Dental implants were originally made of pure titanium surface producing a titanium oxide, strictly bioinert.

Factors contributing to the ultimate success of an implant osseointegration process include physiological conditions of the patient, implant insertion technique, the material the implant is made, implant design and implant surface. Various modifications have been tested of the surface of dental implants to improve the osseointegration capacity and thus a new generation of implants have been developed [1, 2].

The new generation of implants has a high variability of the characteristic features of the area, on both the chemical and structural composition. The surfaces of dental implants have received some changes in order to obtain an enhanced biologic response [3, 4].

A new generation of dental implants that developed a system of dental implants by preparing the surface by etching, sandblasting and immersion in NaCl solution was carried out by the company TAG Dental Implants, Israel. Macro - geometry of these implants increases the total functional area, thus contributing to a favourable distribution of forces effectively reduce stress. The macro-porous surface (mm-40μ) plays a primary role in the stability and long-term mechanical fixation. The surface treatment of these implants is a result of a long experience that had intended to get the best biological response. Chemical and physical properties of the surface of titanium implants such as ionic composition and roughness are the parameters that play a major role in the interaction of implant - tissue. The microroughness surface morphology (40μ- 1μ), achieved by sandblasting followed by etching, increases the contact between the implant and the bone and results in improved mechanical anchorage for better primary stability that promotes cell adhesion. Changing the surface energy at a nano level on the hydrophilic and osteo-conductive surface promotes active ion interactions with blood plasma for a faster osseointegration and distrbution of the contact between implant and bone.

The dental implants also immersed in isotonic NaCl and should improve the ability of hydrophilic surface, reducing the time period of the osseointegration process and increasing the percentage of contact between implant and bone [5, 6].

Currently, studies in oral implantology try to improve the osseointegration process with much faster and better results both in healthy subjects and in patients with various systemic diseases, such as diabetes mellitus [7].

If in addition to the general status of patients with diabetes that has to be rigurously controlled, the implant - bone interaction could be enhanced by various techniques of implant surface preparation, all this would lead to an increased capacity of osteointegration in this population [8, 9].

Inserting a dental implant unrelated to the overall context of the body, the local particularities of the implant recipient site and macro-and micro-structural details of the implant itself can lead to its pseudointegration, accompanied by inadequate biomechanical properties of the interface bone - implant which can result in the failure of the treatment [10].

This study aims to assess the interaction of different dental implants surfaces and peri – implant bone in diabetic rats. With this study we wanted to assess the stages of formation and maturation of peri - implant bone trying to make a correlation between the ability of osseointegration of different dental implants surfaces in diabetic rats.

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Experimental part

Methods and materials

The study was conducted in the Department of Surgery and Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi.

The study protocol was approved by the Ethics Committee of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi. All experimental procedures on animals used in this study were in strict accordance with international ethical regulations.

In this study, were used 60 Wistar rats, male, average weight from 400 - 450 g. Every 10 subjects were housed in each cage and acclimated under the study conditions (temperature 24 ± 1°C, humidity 50% - 60%), the circadian rhythm of 12 h light per day.

They were fed on a laboratory diet strictly provided by Nutrimold (Science, Romania) containing 15% casein, 0.8% of phosphorus, 1% calcium, 70-80% carbohydrate and fat 5% throughout the experiment. Demineralized water was available ad libitum.

The period of acclimatization was carried out for two weeks before the start of the study. After this period, subjects in this study were randomly divided into 6 groups by 10.

The 6 groups were composed as follows:

- Group I - healthy subjects who received implants with surface prepared by sandblasting and etching.
- Group II - healthy subjects who received implants immersed in isotonic sodium chloride.
- Group III - subjects with experimentally induced diabetes that received implants with surface prepared by sandblasting and etching.
- Group IV - subjects with experimentally induced diabetes that received implants immersed in isotonic sodium chloride solution.
- Group V - subjects with experimentally induced diabetes, who received one unit of insulin daily and implants with surface prepared by sandblasting and etching.
- Group VI - subjects with experimentally induced diabetes, who received one unit of insulin daily and implants immersed in isotonic sodium chloride.

Induction of experimental diabetes

Diabetes is obtained by intraperitoneal administration of a single dose of streptozotocin (STZ) 40 mg/kgb, freshly dissolved in 10 mM sodium citrate solution (pH = 4.5).

Rats in the control group received intravenous an amount of sodium citrate proportional to the weight. In order to prevent hypoglycemic shock it is installed at approximately 4-8 hours after administration of STZ, the rats were each given 33% glucose 1 mL intraperitoneal 30 min after the administration of STZ. Subjects were considered diabetic if blood glucose exceeded 200 mg/dL. Tracking blood glucose in diabetic rats will be dynamic, using a glucometer.

Prior to the induction of diabetes, the blood will be harvested from the tail vein to evaluate the blood glucose level, which was carried out using a glucometer. Throughout the experiment, the animals were weight and blood sugar was regularly monitored.

Surgical intervention

After the diabetes mellitus is confirmed, 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 used in this study. The animals were subjected to general anesthesia by intramuscular injection of ketamine 40 mg/kg body weight pentobarbital 20 mg/kg body weight. An incision was made at the distal metaphysis of the left femur and muscle and periosteum were removed.

After exposing the bone, with a guide cutter of 0.8 mm in diameter, the external, medullary and internal cortical of the left femur was trepanned. Sequentially, a trepanation drill was used to expand the gradual opening made in the bone which corresponds to the final diameter and length of the dental implant.

Preparation of the bone was done with drills at a low speed and under a continuous irrigation with saline. There were inserted two sterile implants at the level of the left metaphyseal distal femur in each of all 60 Wistar rats. Surgical wound is sutured with absorbable thread (Vicryl 5-0, Ethicon GmbH, Norderstedt, Germany). To reduce postoperative pain, each subject was administered butorphanol (0.05 mg/kg). Cephalexin antibiotic therapy was performed (15 mg/kg) for 5 days.

Insulin administration

Subjects in groups V and VI individually receive one daily dose of insulin to be administered by subcutaneous injection, for 6 months. The animals in groups I, II, III and IV will receive the same dose of sterile saline instead of insulin.

Electronic microscopy examination

At the end of the study, animals were euthanized by anesthesia ip ketamine 40 mg/kg body weight pentobarbital 20 mg/kg body weight. It caused a quick death, without suffering. The finding of absence of vital signs (respiratory movements, heart beat, reflexes) animals will be dissected to harvest the femur.

Immediately after euthanasia of animals, all samples were provided by forming an incomplete osteotomy with a rotary cylinder at the site where the implant has been inserted, endosulfan and subsequent fracture of the bone fragments in the area of weakness. Subsequently the samples were prefixed 3% glutaraldehyde solution and stored at 40C until the examination by electron microscopy.

After obtaining experimental biological samples, they were examined by electron microscopy scanning using a microscope type Tescan Vega II LMU an accelerating voltage of 30 kV electron beam being used, scoring a 30 A 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. It was considered that the most representative electron microscopy evaluation stage is 180 days from the insertion of endosseos implants.

Results and disscusions

Clinical findings

The overall postoperative wound healing after implant placement was excellent. No tissue dehiscencesor visible infections were noted during the study period, and 112 from 120 placed implants were available for histological analysis.

Results of serum glucose levels

Before surgery, serum glucose levels were determined at 72 hours and one week after the induction of diabetes. Subsequently, the serum glucose was determined once a week. These measurements were made with a glucometer. As can be seen in figure 1, serum glucose

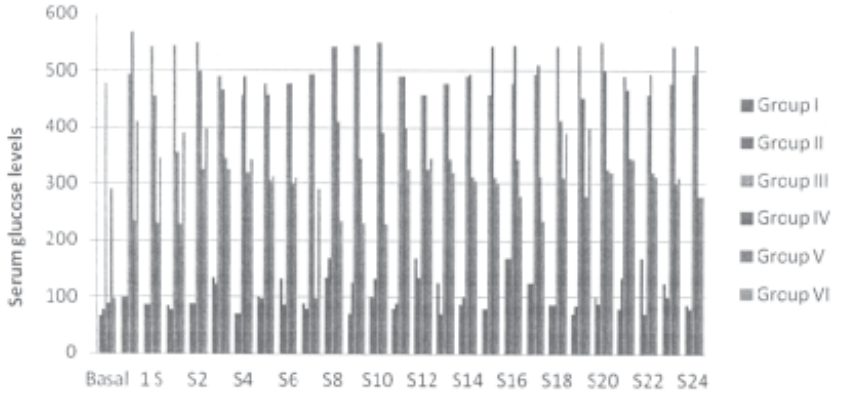

Fig. 1. The evaluation of dynamic serum glucose levels before the induction of diabetes, 72 h and 1 week after the injection of streptozotocin, and post-operative weekly, for 6 months

levels are higher in the diabetic subjects (groups II, IV, V and VI) in comparison with the control subjects (group I and II) during the 6 months of the experiment.

Insulin treatment significantly reduced the elevated blood glucose in subjects in group V and VI, which received one unit of insulin daily. In contrast, subjects in groups III and IV there is a tendency to increase serum glucose levels because they have not benefited from a controlled glycemic status by insulin therapy. These data suggest that the experimental conditions are safe.

Results of the analysis of electron microscopy scanning for group I

Scanning electron microscopy of surface samples with endosseous implants prepared by sandblasting and etching (Tag Dental Implants, Tag Medical, Israel) revealed that the implant was covered with new bone formed over a wide area in direct contact with the implant.

Results of the analysis of electron microscopy scanning for group II

Analysis by electron microscopy of samples of endosseous implants immersed in isotonic sodium chloride solution (Tag Dental Implants, Tag Medical, Israel) showed that the implant surface was covered with newly formed bone tissue over a large areain contact with the implant.

In addition, in these endosseos implants, a larger number of osteoblasts was found to adhere to the surface of the implant. Surface roughness at this level was the main determinant of nonosseos grip for this type of implant.

Results of the analysis of electron microscopy scanning for group III

Of the 20 endosseous implants inserted at the distal metaphysis of the left femur, eight were not osseointegrated and were excluded from further investigations.

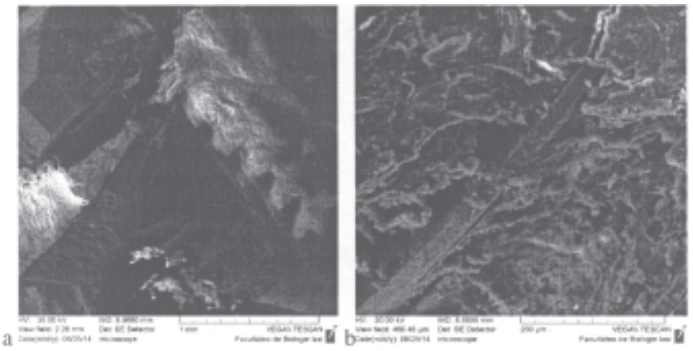

Fig. 2. SEM image of dental implants with surface prepared by sandblasting and etching in healthy subjects (group I)

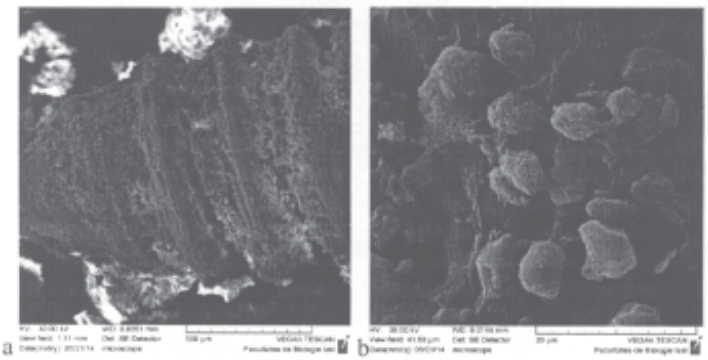

Fig. 3. SEM images of dental implants immersed in isotonic sodium chloride in healthy subjects (group II)

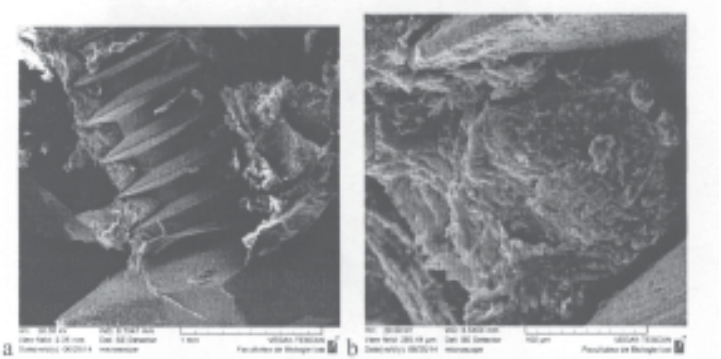

Fig. 4. SEM images of dental implants with surface prepared by sandblasting and etching in uncontrolled diabetic subjects (group III)

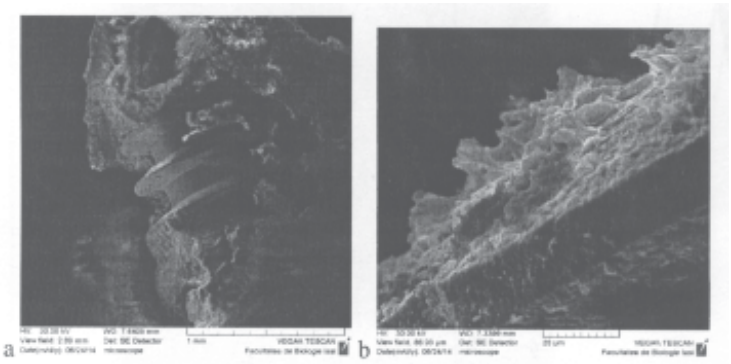

Fig. 5. SEM image of dental implants immersed in isotonic sodium chloride in uncontrolled diabetic subjects (group IV)

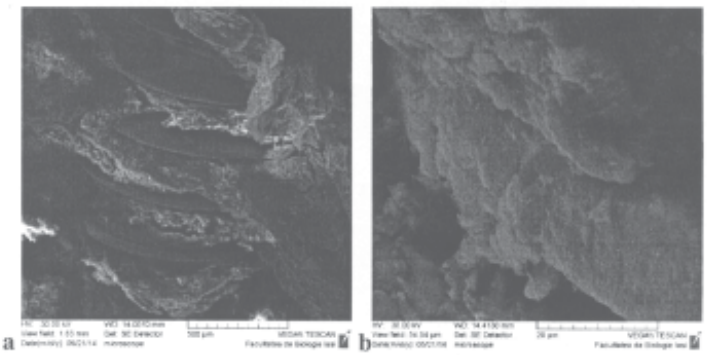

Fig. 6. SEM image of dental implants with surface prepared by sandblasting and etching in controlled diabetic subjects (group V)

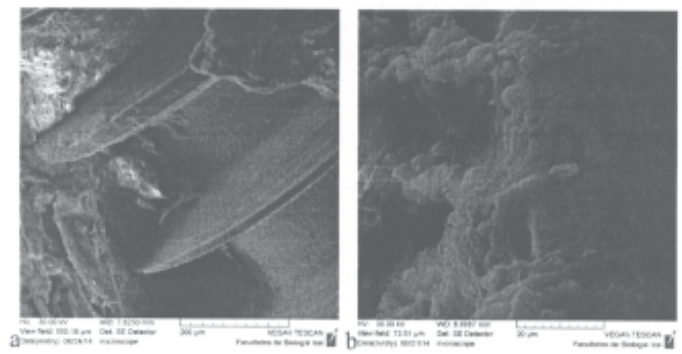

Fig. 7. Electron - microscope scanning of dental implants immersed in isotonic sodium chloride in controlled diabetic subjects (group VI)

In endosseous implants prepared by sanding the surface and etching (Tag Dental Implants, Tag Medical, Israel), examination of the interface bone - implant electron microscopy revealed the presence of a small amount of mature functional bone held in contact with the entire length of the implant. A lack of uniform attachment of bone to implant was also found in most of the examined samples.

Results of the analysis of electron microscopy scanning for group IV

For samples with endosseous implants immersed in isotonic sodium chloride (Tag Dental Implants, Tag Medical, Israel) at a macroscopic examination one may observe that they were covered with bone only at the apical, and the microscopic images detected the presence of the newly formed bone with a low degree of differentiation.

Results of the analysis of electron microscopy scanning for group V

Macroscopic examination of endosseous implant surface samples prepared by sandblasting and etching (Tag Dental Implants, Tag Medical, Israel) revealed that they were fully covered by bone and the microscopic images detected the presence of newly formed bone with a low degree of differentiation.

Results of the analysis of electron microscopy scanning for group VI

Scanning electron microscopy of samples with endosseous implants immersed in isotonic sodium chloride (Tag Dental Implants, Tag Medical, Israel) showed that the implant surface was covered with new bone well formed, represented quantitatively which began to develop lamellar appearance between blades and many red blood cells can be observed.

Diabetes adversely affects bone regeneration around dental implants with a low percentage of contact between implant and bone. On the other hand, the insulin therapy allows the maintenance of healthy subjects with similar results.

Regarding the periimplantar tissue bone, literature presents controversial results. Bone regeneration in diabetic animals was impaired compared with the healthy ones [11].

Our study has demonstrated the negative effect of hyperglycemia on the osseointegration process, which is in agreement with other studies [12-15].

Descriptive results of this study are consistent with other histologic studies, have shown that diabetes may be associated with low bone regeneration around dental implants in diabetic rats compared to the non-diabetics [12, 14, 16-18]

Insulin glycemic control can balance, accurate changes in the bone caused by diabetes mellitus and maintain a similar bone density [19-21].

Our study agrees with these findings because the aministering of balanced insulin and glycemic control in subjects in groups V and VI, and osseointegration of dental implants was significantly greater than those in groups III and IV, which did not receive insulin therapy.

The influence of surface roughness of dental implants on initial cell responses has not yet been fully explained. However, several authors argue that a high surface roughness, achieved by the texturing techniques, such responses would help. Recently, some researchers have suggested that osteoblasts have a higher affinity for surfaces with a high degree of micro-roughness.

In our study, the dental implnats surfaces have undergone a process of reduction, i.e. acid etching, which provided relatively low roughness values , similar to those suggested by some authors as the ideal [22].

More than one assessment method must be used to characterize the surface of dental implants because no isolated technique can faithfully reflect topographic findings. There are a variety of techniques for bi-and tri-dimensional measurements to characterize the surface. The degree of cell proliferation was lower in acid etched and sandblasted surfaces compared to surfaces that have received treatment with isotonic NaCl. It is possible that roughness can promote cell differentiation of the osteogenic lineage cells adhered, despite the weak findings in terms of cell proliferation. Similar results comparing the different levels of surface roughness produced by the abrasive particles in the air, found aassociation of the increase in alkaline phosphatase activity and total protein content with lower degrees the proliferation of the cells [23].

A possible limitation of this study relates to the difficulty to compare our results with other studies available in the literature, the lack of characterization of the surfaces tested, the knowledge of cell and tissue responses, obtained by using different surface treatment options for diabetic rats. Some in vitro studies have found an association between the increased surface roughness and improve the proliferation of the cells [24-27].

However, in this study, a small cell proliferation index has been observed on surfaces with a higher roughness compared to the less rough surface.

The dental implant surfaces treated with isotonic NaCl were osseointegrated with a high level compared with the others in diabetic rats. The preparation in advance of both the metabolic status of diabetic patients and improving the osseointegration of dental implants surface preparation can help you get much better results in this population.

Conclusions

In conclusion, experimentally induced diabetes adversely affects bone around dental implants because they were negative in terms of cell adhesion to the surface of implants and implant-bone contact between the subjects who did not receive glycemic control in insulin-therapy. Insulin therapy prevented these modifications of the peri – implant bone, osseointegration, thus realizing it similar to the control group. The results obtained in this study suggest that dental implants surfaces prepared by acid etching and sandblasting or have received treatment with isotonic NaCl can accelerate osseointegration in diabetic rats, a finding which may become by further studies to be applied to diabetic patients.

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