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EVALUATION OF BONE TISSUE RESPONSE IN THE MEDIUM TERM AFTER INSERTION OF A TITANIUM IMPLANT WITH A SURFACE FUNCTIONALISED WITH TITANIUM SIOXIDE NANOTUBES

EVALUAREA REACȚIEI ȚESUTULUI OSOS PE TERMEN MEDIU, DUPĂ INSERAREA IMPLANTULUI DIN TITAN CU SUPRAFAȚĂ FUNCȚIONALIZATĂ CU NANOTUBURI DIN DIOXID DE TITAN

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ABSTRACT

Titanium and its alloys are the materials of choice used in the field of maxillofacial, dental, and orthopaedic restorations. Titanium is a biomaterial that, upon contact with any oxygen-containing element (air, water), spontaneously forms a protective amorphous coating with a thickness of less than 10 nm. Currently, new methods are being sought to enhance the osseointegrative capacity of titanium implants through surface improvements using processes such as sandblasting, acid etching, the addition of bioactive coatings, and anodisation. The aim of this study was to histologically evaluate the bone tissue response in the medium term after the insertion of commercially pure grade 4 titanium implants with acid-etched surfaces, compared to a surface organised into titanium dioxide nanotubes.The titanium dioxide nanotube surface was obtained through the process of electrochemical oxidation (anodisation).

Key words: coating surface of dental implant, dental implant functional surface, osseointegration

REZUMAT

Titanul și aliajele acestuia sunt materiale de elecție utilizate în domeniul restaurărilor maxilo-faciale, dentare și ortopedice. Titanul este un biomaterial care în contact cu orice element care conține oxigen (aerul, apa) formează spontan un înveliș de protecție cu structură amorfă cu o grosime sub 10 nm. În momentul actual se caută noi metode pentru a crește capacitatea osteointegrativă a implanturilor din titan prin îmbunătățiri ale suprafeței acestora utilizându-se procedee de sablare, decapare cu acid, adaosuri de învelișuri bioactive și prin anodizare. Scopul acestui studiu a fost acela de a evalua, din punct de vedere histologic,reacția țesutului osos pe termen mediu, după inserarea implanturilor din titan tip pur comercial grade 4 cu suprafață decapată cu acid, comparativ cu o suprafață organizată în nanotuburi din oxid de titan. Suprafața cu nanotuburi din oxid de titan a fost obținută în urma procesului de oxidare electrochimică (anodizare).

Cuvinte cheie: suprafață de acoperire a implantului dentar, suprafață funcțională a implantului dentar, osteointegrare

Titanium is a biomaterial of choice in the dental and orthopaedic implantology industry (3). When in contact with air, water, or any oxygen-containing element, this material instantly forms a titanium oxide coating with an amorphous structure (10, 4, 22, 21).

Titanium falls into the category of biomaterials because it meets the primary properties of interest, such as biocompatibility, mechanical strength, osseointegration, and also possesses antibacterial properties (15, 23). The term osseointegration was first used by Albrektsson in 1981. This term referred to the functional and structural relationship between the surface of the implant and the tissue in which it is inserted (6).

This process of osseointegration is directly influenced by a series of factors involving:

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- The implant (design, surface, biocompatibility of the constitutive material, storage conditions, sterility, etc.)
- The implantologist (technique applied, surgeon's skills), adherence to working procedures, treatment planning, optimal selection of the type of implant according to the type of bone, accurate anticipation of occlusal forces, etc.
- The quality of the bone in which the implant is inserted (6).

Thus, the characteristics of the implant surfaces and the quality of the bone determine the interface between bone and implant (8). Elias C.N. in 2011, and later Velloso G. et al. in 2019, observed that if the implant is positioned in spongy bone, the bone/implant contact is approximately 50%, while if the bone is compact, the contact between it and the implant can be up to 90%.

One of the essential roles on which the long-term success of the implant's osseointegration depends is played by the implant surface (2). Optimising the surfaces of dental implants is recognised as a key element for improving implant osseointegration (16).

The physicochemical properties of the surface and the response of the bone tissue in which the implant is inserted determine the biological anchorage of the implant (13, 14). Regardless of the physicochemical method applied to the implant surfaces, they can be hydrophilic or hydrophobic (19, 7). Rough surfaces (such as those that are sandblasted, acid-etched, or anodised) are hydrophilic, compared to smooth structures that are more hydrophobic (7, 12). Hydrophilicity favours the interactions of the implant surface with cells and biological fluids, allowing good wettability of the surface (7, 20) and implicitly superior cell adhesion.

Electrochemical anodic oxidation (anodisation) is used to grow a uniform oxide layer on metals and increase its thickness. This surface functionalisation of the implant has proven to play an excellent role in the biocompatibility of implant metals (10).

Currently, the most widespread surface of commercial dental implants is the rough surface obtained by sandblasting with coarse-grained corundum and then acid-etched (SLA), which provides the surface with hydrophilic qualities (19).

In a study on the biocompatibility of titanium dioxide (TiO_2) surfaces, a surface organised into TiO_2 nanotubes was obtained through anodisation. The outer diameter of a nanotube is 50-60 nm, with a wall thickness of 10-15 nm and a height of 100-200 nm. This way, the exposed titanium dioxide surface area is expanded approximately sixfold within the internal environment of the body.

The present study refers to the comparative observation of the peri-implant bone tissue response post-insertion of two types of implants with hydrophilic surfaces, namely Ti CP grade 4 implants with an SLA surface and implants with an anodized surface functionalized with titanium dioxide (TiO_2) nanotubes. All other characteristics of implants used in the study were identical (material, architecture, dimensions, etc).

MATERIALS AND METHODS

The biological materials used for this experiment were represented by 10 adult New Zealand white albino rabbits (9-10 months old), males, intact, with a body weight between 3.110 and 3.890 kg at the start of the test. For this experiment, 24 customised implants with an SLA surface and 24 implants with a surface functionalised with TiO_2 nanotubes were used. The test implant, named T3 by the manufacturer, consists of a cylindrical screw made of Ti CP Gr.4, with a length of 4.2 mm and a thickness of 2 mm, having 5 threads and a functionalised surface.

The control implant, named T2 by the manufacturer, consists of a cylindrical screw made of Ti CP Gr.4, with a length of 4.2 mm and a thickness of 2 mm, identical in size to the test samples.

The T2 and T3 implants were subjected to gamma radiation sterilisation according to specific protocols and kept sealed until their intended use.

The Commercial Purity Titanium (Ti CP) Gr4 implants (98.4% purity – supplier Dynamet USA) were mechanically processed on a CNC lathe, Citizen Cincom L20 type. All implants underwent the sandblasting process. Sandblasting was performed in an automatic sandblasting system, Dentix Microblast type, using electrocorundum(Al2O3) as the blasting material.

After sandblasting, the T2 implants were subjected to acid etching. For the etching process, hydrochloric acid 36.5-38% (Sial Trading) and sulphuric acid 94-96% (Sial Trading) were used. The anodised implants (T3) were produced on the automated surface treatment and washing line, Dentix Nano Line type.

Finally, the implants were washed on the same line, following washing programs with special detergent and successive rinses with ultrapure water, with conductivity levels of 0-2 µS/cm, followed by drying in HEPA (High Efficiency Particulate Air) filtered air.

The guide for conducting this experiment was taken from the standard SR EN ISO 10993 - ISO 10993-6:2016 Annex C Tests for local effects after implantation in bone. Also considered were parts 2 (Biological evaluation of medical devices Part 2: Animal welfare requirements), 12 (Biological evaluation of medical devices, Part 12: Sample preparation and reference materials), and 15 (Biological evaluation of medical devices Part 15: Identification and quantification of degradation products from metals and alloys) from the 10993 standard series. The animals were numbered according to Table 1 to maintain better data records.

Table 1
Numbering and identification of animals subjected to the study, according to testing duration

No crt	Animal Identification Number	Testing Duration
1	*I1M*	*180 days*
2	*I2M*	*0*
3	*I3M*	*150 days*
4	*I4M*	*120 days*
5	*I5M*	*90 days*
6	*I6M*	*60 days*
7	*I7M*	*0*
8	*I8M*	*0*
9	*I9M*	*0*
10	*I10M*	*30 days*

During the acclimatisation period (minimum 10 days) and the experiment, the animals were housed in individual cages made of easily washable, durable material, with a perforated floor to prevent contact with excrement. The space was properly ventilated at a temperature of 17-23°C. Water and food were provided ad libitum throughout the acclimatisation period (10 days) and the entire duration of the experiment (30-180 days), except for the 24-hour period before the surgical intervention.

Animals subjected to the implantation surgery had three T2 implants and three T3 implants inserted into their tibiae. The following implantation protocol and

postoperative treatment were used:

• The surgical kit is prepared, the physiodispenser, physiological solution source, and specific surgical instruments are set up.

• Animals are clinically evaluated, weighed, and their temperature is taken, with data being recorded.

• Animals are restrained.

• Sedation is performed by intramuscular (IM) administration of a mixture of medetomidine (Domitor) 0.25 mg/kg and ketamine (Ketadorm) 15 mg/kg.

• The selected area – the tibia – is prepared (hair is shaved, the area is disinfected, and a sterile surgical field is applied).

• Anaesthesia is supplemented as needed by inhalation of isoflurane (Isoflurane USP) 0.5-2.5%.

• A 5-7 cm incision is made on the ventral side of the tibia.

• Muscle fasciae are debrided.

• The tibia is exposed by periosteal stripping.

• The bone is drilled using a 2 mm drill fixed to a physiodispenser (SurgicXT Plus by NSK Dental) set at 1200-1500 rpm, under physiological solution irrigation.

• Implants are inserted by screwing.

• Muscle fascia is sutured with absorbable Bicril 0.2 thread.

• The skin is sutured with 0.2 silk.

• Atipamezole (Antisedan) 0.5 mg/kg is administered IM.

Postoperative care includes:

• Oxygen therapy;

• Antibiotic therapy - Enrofloxacin 5%, 1 ml, subcutaneously for 3 days;

• Analgesic therapy with Ketoprofen 5%, 0.2 ml, ubcutaneously, post-implantation;

• Local application of Neocaf spray.

Samples were collected after euthanising the animals. For euthanasia, animals were sedated with a mixture of medetomidine (Domitor) 0.5 mg/kg and ketamine (Ketadorm) 15 mg/kg, followed by the administration of 2 ml of T61 intrapulmonary.

The collected samples consisted of bone sections (tibia) with the corresponding implant. Thus, 3 bone tissue samples with type T2 implants and 3 bone tissue samples with type T3 implants were collected from each animal at each testing time (one animal per testing time). The chosen testing times were: 30, 60, 90, 120, 150, and 180 days post-implantation.

After collection, the samples underwent demineralisation. This required immersing the bone tissue containing the implant in an Osteodec decalcifying solution for 48 hours. After demineralisation, the implants were removed, and the bone tissue was embedded in paraffin blocks, then sectioned with a microtome into 5-micron thick sections, stained with haematoxylin-eosin, and examined under an optical microscope at 200X and 400X magnification.

For each collection time, the samples were comparatively evaluated: samples containing type T2 SLA implants and samples containing type T3 implants with a surface functionalised with titanium nanotubes. The collected samples were histologically evaluated for bone tissue reaction post-insertion of the titanium implant, with 3 bone tissue samples collected per tibia along with the inserted implant.

RESULTS AND DISCUSSIONS

Following the sandblasting and acid etching processes of the T2 implants, an SLA-type rough surface with hydrophilic capacity was obtained (Fig. 1).

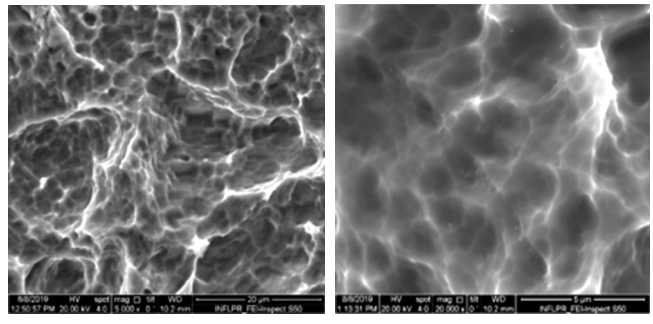

Fig. 1. T2 implant surface (SLA) observed at SEM magnification 5,000X on the left and 20,000X on the right

Following the anodisation process, with a complex course of voltage and electric current, a surface structured in TiO_2 nanotubes (Fig. 2) with hydrophilic capacity is obtained.

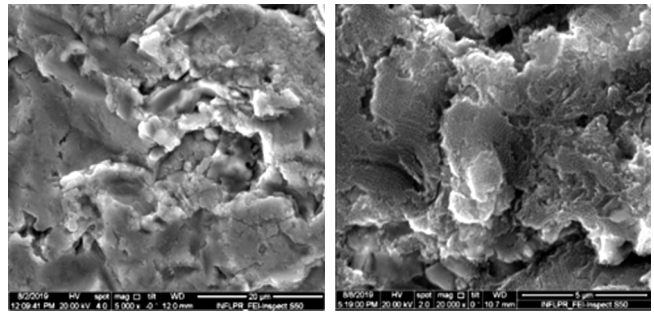

Fig. 2. T3 implant surface (surface functionalised with titanium nanotubes) observed at SEM magnification 5,000X on the left and 20,000X on the right

The implants were intraoperatively inserted as follows: T2 type on the right limb (control) and T3 type implants on the left limb (test material), in a number of 3 pieces on each limb, totalling 6 implants per animal. During the surgical procedure, animal I2M was excluded from the study because it experienced cardio-respiratory shock at the time of anaesthesia, preventing the surgical intervention. Subject I8M was excluded from the study on day 5 post-implantation due to self-mutilation. Animal I9M was excluded from the study because on post-operative day 3, it removed the skin sutures, contaminating the area and causing secondary healing. Rabbit I7M was not subjected to surgical intervention and was kept as a reserve animal in case the remaining animals in the study were insufficient to cover the testing times. After collecting the samples

from the remaining animals in the study, following histological evaluation of peri-implant bone tissue samples, the following aspects were observed (Table 2):

For the 30-day post-implantation collection:

• In the bone tissue containing T2 implants, a slight periosteal reaction was observed, with infiltrates of mononuclear cells, fibroplasia, and neoformation capillaries. Granulation tissue was observed around the implant cap.

• In the bone tissue containing T3 implants, neoformation capillaries and granulation tissue were identified around the implant collar.

For the 60-day post-implantation collection:

• Adequate osteointegration of T2 implants was observed without inflammatory reaction, and haematogenous marrow was replaced with adipose tissue.

• The bone tissue bordering the spirals of T3 implants showed evident calcium salt deposits, a normal aspect in the ossification process. Active osteoclasts were observed in the Howship's lacunae.

For the 90-day post-implantation collection:

• Fine granular calcium salt deposits were observed around the spirals of T2 implants, along with Howship's lacunae typical of bone remodelling. Uniformly distributed Volkmann's canals with unchanged appearance were noted.

• Near the T3 implants, the bone tissue exhibited normal morphology without periosteal reaction.

For the 120-day post-implantation collection:

• Proper bone modelling was observed around T2 implants with normal histological characterisation and no inflammatory reactions. Volkmann's canals were uniformly distributed and unchanged.

• There were no peri-implant reactions observed around T3 implants. The bone marrow showed typical conjunctive-adipose tissue without specific elements of myeloid or lymphoid cell lines.

For the 150-day post-implantation collection:

• The bone tissue bordering the spirals of T2 implants showed evident calcium salt deposits, indicating ongoing ossification. No myeloid or lymphoid cells were observed in the bone marrow.

• Near the T3 implants, the bone tissue morphology remained unchanged.

• For the 180-day post-implantation collection:

• Perfect osteointegration was observed in the bone tissue containing T2 implants, with normal bone appearance and complete bone remodelling.

• T3 implants also showed perfect osteointegration with normal bone appearance and total bone remodelling.

The field of biomaterials engineering is continuously advancing, with ongoing research aimed at discovering new biomaterials or improving the structure, architecture, surface or function of existing ones (17).

The surface functionalised with TiO_2 nanotubes offers promising perspectives in implantology, demonstrating biocompatibility, osteointegration, and regenerative capabilities (1, 9).

This innovative nanostructured surface can significantly enhance the performance and durability of medical implants. Bone formation is the reaction that confirms osteointegration. In the current study, similar to other experiments, the biocompatibility and osteointegration of implants with a surface functionalised with titanium nanotubes were demonstrated. Both types of implants (Ti CP grade 4 with SLA surface and anodised implants functionalised with titanium dioxide nanotubes) achieved complete osteointegration at 180 days.

CONCLUSIONS

Histological exam revealed the similarity in osteointegration stages of T2 and T3 implants across all tested individuals. In the initial stages of osteointegration (30-day collection), a superior tissue response was observed for T3 implants compared to T2 implants: a slight periosteal reaction with mononuclear cell infiltration was seen with T2 implants, while this evolution was not observed with T3 implants. In the medium term, after the insertion of CP grade 4 titanium implants with a titanium oxide nanotube surface, the bone tissue showed normal specific organisation. For both types of implants, the osteointegration process

Table 2

Highlighting and Systematising Post-Implant Tissue Reactions

Period of Collection	Implant type	Inflammatory Reaction	Neovessel Formation	Granulation Tissue	Calcium Deposits	Presence of Howship's lacunae	Osseointegration 100%
30 days	T2	x	x	x	-	-	-
	T3	x	x	x	-	-	-
60 days	T2	-	x	-	x	x	-
	T3	-	x	-	x	x	-
90 days	T2	-	-	-	x	x	-
	T3	-	-	-	x	x	-
120 days	T2	-	-	-	x	-	-
	T3	-	-	-	x	-	-
150 days	T2	-	-	-	x	-	-
	T3	-	-	-	x	-	-
180 days	T2	-	-	-	-	-	x
	T3	-	-	-	-	-	x

was complete on day 180 post-insertion. The titanium oxide nanotube-organised surface is at least as biocompatible and osteointegrative as the SLA surface of CP grade 4 titanium implants, with no measurable differences between the two types of implants.

Ethics Statement

In terms of animal protection used in this experiment, the provisions of law no. 43/11.04.2014 regarding the protection of animals used for scientific purposes and the order of ANSVSA (National Sanitary Veterinary and Food Safety Authority) 97/1.09.2015 for the approval of the sanitary veterinary norm regarding the procedure for sanitary veterinary authorization of user, breeder, and supplier units of animals used for scientific purposes were followed, as well as recommendations issued by FELASA (Federation of European Laboratory Animal Science Association) regarding animals used in testing. Considering the request and documentation registered under no. 3228/13.02.2019 by the National Institute for Research and Development in Physics and Nuclear Engineering Horia Hulubei, favorable approval (no. 56/11.02.2019) from the ethics committee and authorization of the research project by the Veterinary and Food Safety Directorate Bucharest (Project Authorization no. 5/22.02.2019) were obtained. For the insertion of implants, administration and application of samples, clinical monitoring, and sample collection, all measures were taken to reduce animal suffering.

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Author contributions

All authors made substantial contributions to all of the following: (1) the conception and design of the study, acquisition and interpretation of data; (2 and 3) final approval of the version to be submitted; (4) histopathological investiation and (5) drafting the article or revising it critically, scientific coordination.

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