JOURNAL OF OSSEOINTEGRATION
periodontics and prosthodontics

Anodization as a promising surface treatment for drug delivery implants and a non-cytotoxic process for surface alteration: a pilot study

M. F. KUNRATH[1*], N. PENHA[2], J.C. NG[3]

[1] Dentistry University, School of Health Sciences, Pontifical Catholic University of Rio Grande do Sul (PUCRS) - Av. Ipiranga, P.O. Box 6681, 90619-900, Porto Alegre, Brazil
[2] Implante Institute, Av. Rio Branco 26, P.O Box 20090001, Rio de Janeiro/RJ, Brazil
[3] The University of Queensland, Queensland Alliance for Environmental Sciences, PACE Building, 20 Cornwall Street, Woolloongabba, Qld 4102, Australia

TO CITE THIS ARTICLE
Kunrath MF, Penha N, Ng JC. Anodization as a promising surface treatment for drug delivery implants and a non-cytotoxic process for surface alteration: a pilot study. J Osseointegr 2020;12(1):XXXX.

DOI 10.23805 /JO.2020.12.01.01

ABSTRACT

Aim Surface treatments use industrial processes in which surface contamination can occur. In this context, this study aimed to demonstrate a surface treatment process, from laboratory samples and clinical implants, named anodizing, analyze their tendencies to surface contamination as well as their properties.

Materials and Methods Laboratorial samples of pure titanium were anodized. Investigated by scanning microscopy (SEM), dispersive energy spectroscopy (EDS) and wettability tests. Four implant systems available in the current market were chosen by different surface treatments (anodizing, double acid etching and particle blasting) and investigated by SEM/EDS.

Results Laboratory samples showed a nanomorphology surface, free of contaminants and good liquid/surface interaction. The implant system with anodization treatment did not present elements outside the standards. However, the implants treated with acid attack and blasting were found different chemical elements like aluminum and magnesium.

Conclusions Anodizing proved to be a contaminant-free surface treatment both in the laboratory and clinical implants. In addition, its promising property of owning TiO2 nanotubes suggests an inherent evolution to biomedical implants for drug delivery systems other than all surface treatments developed to date.

KEYWORDS: Dental implants; Surfaces; Anodization; Contamination; Drug delivery.

INTRODUCTION

Surface treatments and drug delivery systems are currently widely discussed by researchers in the field of biomedical implantology (1-2). Surface treatment technologies evolve rapidly and always aim for better performance for intended clinical use (3). In biomedical implants made from Titanium (Ti), a surface treatment process involving biomedical engineering, pharmacology, and implantology is generally known as electrochemical anodization (4).

Contemporary anodization process promotes the surface treatment with nanotubes (TNTs), which allows the surface loaded with numerous nanoparticles (NPs) or alternatives of drugs (4-6). Furthermore, because it is a surface nanotechnology, studies have reported that this treatment method has the best ability in promoting cell interaction with bone cells during the process of adhesion and proliferation (7-8) after implantation, as well as a lower adhesion and proliferation reaction of bacteria that may cause infections in biomedical implants (9).

One of the concerned factors in surface treatments for biomedical implants is the contamination of non-biocompatible chemical elements during the manufacturing process (10). Chemical elements such as aluminum and aluminum oxide, nickel, copper, vanadium, among others have been reported (10-11) as contaminants and their potential cytotoxicities. These are usually found after superficial treatments with different methodologies or in implants made with impure titanium alloys (10,12). This surface cytotoxicity generated by non-biocompatible elements is of health concern and should be avoided for biomedical implants.

This study aims to screen samples made in the laboratory as in commercially available clinical implants that used the surface treatment by anodization using surface atomic reading of the samples and surface characterization. A current view of the promising studies involving anodized surfaces with TNTs and their various possibilities of functionalization is also discussed.

ASTM F67 Standarts						
ASTM F67 pure Titanium grade I						
Chemical Element	Nitrogen (max.)	Carbon (max.)	Hydrogen (max.)	Iron (max.)	Oxygen (max.)	Titanium
Maximum allowed percentage (%)	0.03	0.08	0.015	0.2	0.18	Balance
ASTM F67 pure Titanium grade II						
Chemical Element	Nitrogen (max.)	Carbon (max.)	Hydrogen (max.)	Iron (max.)	Oxygen (max.)	Titanium
Maximum allowed percentage (%)	0.03	0.08	0.015	0.3	0.25	Balance
ASTM F67 pure Titanium grade III						
Chemical Element	Nitrogen (max.)	Carbon (max.)	Hydrogen (max.)	Iron (max.)	Oxygen (max.)	Titanium
Maximum allowed percentage (%)	0.05	0.08	0.015	0.3	0.35	Balance
ASTM F67 pure Titanium grade IV						
Chemical Element	Nitrogen (max.)	Carbon (max.)	Hydrogen (max.)	Iron (max.)	Oxygen (max.)	Titanium
Maximum allowed percentage (%)	0.03	0.08	0.015	0.5	0.4	Balance

TABLE 1 ASTM Standard F67 with chemical elements and maximum quantity.

MATERIALS AND METHODS

Preparation of laboratory samples and anodization process

for the samples preparation by the anodizing process, a pure grade II titanium plate (Baumer, São Paulo, Brazil) was cut into 10 discs (6 mm diameter and 1mm thick), washed with acetone, deionized water (DI) and dried in a vacuum chamber (Quimis®, São Paulo, Brazil) at a pressure of 0.1mPa for 2 hours. For electrochemical anodization of samples, the samples were submerge in a solution of composed of ethylene glycol , 0.5% NH_4F (ammonium fluoride), 10% DI in an ultrasonic bath with a controlled voltage (40V) and a temperature of 15 ° C. The titanium samples were used as anode and a platinum plate as a cathode. The anodized disc is then washed with a 70% alcohol wash and DI then dried.

Commercial implants with different surface treatments

Several commercially available implants were chosen to investigate the surface treatment under a real clinical condition. These included sterilized implants of Nobel Biocare, Replace, Switzerland - 5 mm x 11.5 mm - lot 491738 (anodizing) (13), Bionnovation, Biodirect, Brazil - 4.0 mm x10 mm - lot 051645 (acid etching), Implacil de Bortoli, UNII Cônico HI, Brazil - 3.5 mm x 7mm - lot 6034566 (aluminum blasting) and Systhex, Classic-ci, Brazil - 4.0mmx8.5mm - lot 140277 (aluminum blasting) registered as Pure Titanium by International Standards Worldwide Organization (ASMT F67).

Surface characterization of laboratory samples

For characterization of the surface after anodization, a scanning electron microscope (SEM, Inspect F50, Tokyo, Japan) was used to verify surface morphology, Dispersive Energy Spectroscopy (EDAX, New Jersey, USA) was used to distinguish the chemical elemental composition of the surface, and a Goniometer - Contact Angle Measure (Phoenix 300, SEO, Kosekdong, Korea) was used to verify the level of wettability of the surface soon after the anodization.

Characterization of commercial implants

To investigate the surface of commercial implants an electron microscope (SEM, Fei Quanta 250, MA, USA) coupled with an EDS system (EDAX, New Jersey, USA) with 15KV was used for the surface treatment analysis. The implants were removed from the sterilized packaging with titanium tweezers on a double sided adhesive tape and immediately submitted for analysis under the microscope.

For the detection of elements in varied gray scale, EDS was applied. In accordance with the ASTM Standard F67, only the nitrogen, carbon, hydrogen, iron, oxygen and titanium were considered as shown in Table 1. All implants were analyzed throughout their structure including the head, body, internal, external and apex threads.

RESULTS AND DISCUSSION

Laboratory Samples

The laboratory sample is showing a surface nanomorphology composed of TNTs (Fig. 1, panel 1). Its atomic surface analysis demonstrated the maintenance of titanium purity after the anodizing process showing no apparent surface contamination as shown by EDS (Fig 1, panel 2). In addition, the wettability test revealed a good

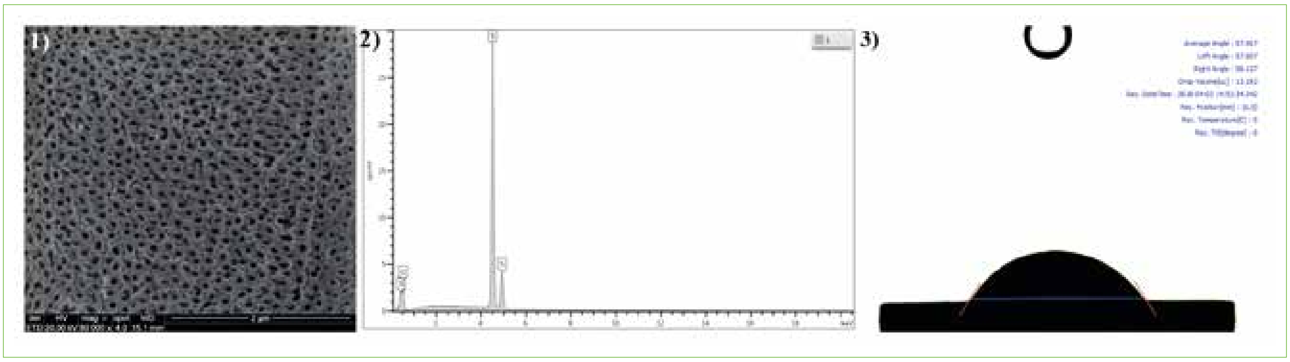

FIG. 1 Surface properties of anodized laboratory samples. Nanomorphology (1), surface purity (2) and wettability (3).

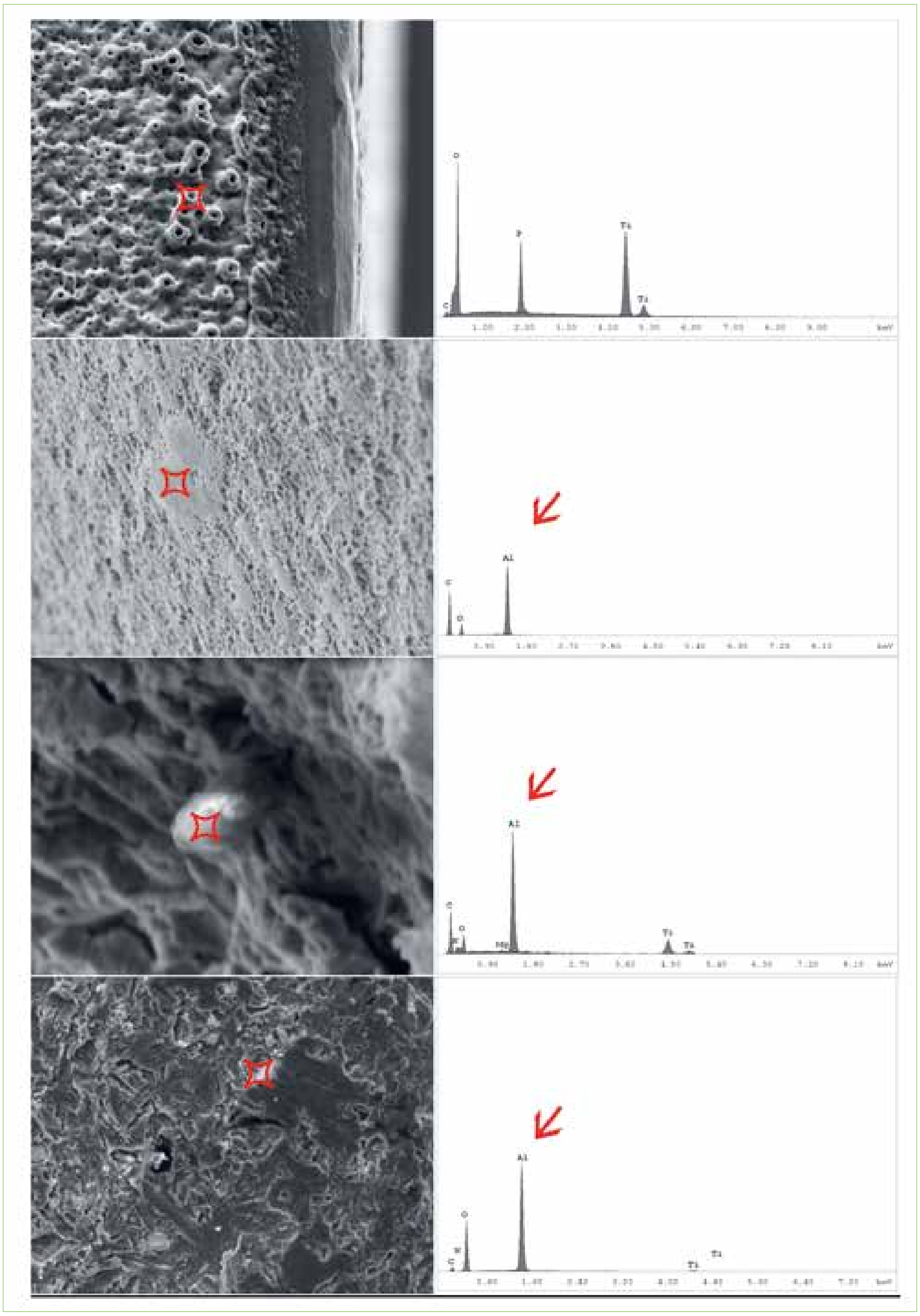

FIG. 2
EDS analysis in commercial implants. Anodizing treatment showing spectrum without unexpected contaminants (A). Acid etching and particulate blasting treatments showing unexpected aluminum (B, C, D) and magnesium (C) residual peaks (red arrows) on implants of pure titanium.

Drug delivery	Application	References
Antibiotics	Significant reduction of bacterial adhesion.	[14-15]
Metals Nanoparticles	Reduction of bacterial adhesion and proliferation.	[16-17]
Anti-inflammatories	Anti-inflammatory effect and cell proliferation.	[18-19]
Natural Drugs	Osteogenic improvement in vitro and in vivo.	[20]
Specific Proteins	Promotes cells expression and proliferation.	[21-22]
Antimicrobial Peptides	Antimicrobial effect.	[23]

TABLE 2 Variety of functionalization in anodised surfaces.

condition of the surface and liquid interaction, with no hydrophobic characteristics (Fig 1, panel 3). Expected characteristics for better cell / surface interaction.

Commercial implants

The microscopic analysis of commercial implants with anodizing treatment showed a morphology with micro and nano pores as can be visualized in Figure 2-A and a uncontaminated surface of elements, confirming titanium purity and treatment without contamination (Fig 2-A). However, implants with other surface treatments show elements such as aluminum (Al) and magnesium (Mg) (Fig 2- B, C, D), that exceed the maximum concentrations as stipulated by the ASTM. Suggesting some contamination or traces of elements during the process of surface treatment or preparation of the implant.

Perspectives for drug delivery in anodized biomedical implants

As presented in this study the electrochemical anodization process can produce surfaces that can be used in biomedical implants without surface contaminations and with functionalization possibilities (nanotubes). The parameters defined in the anodization process will define the shape and length of the tubes as (4,6): solution used, treatment time, temperature, voltage, among others. Thereby, the incorporation of drugs, nanoparticles (NPs), and proteins is totally viable and very promising.
To demonstrate the promising technology and its current status, Table 2 shows some works with specific surfaces incorporations made by anodization for the use of a drug delivery system in biomedical applications.
Surface nanomorphologies are explored by researchers with the objective to improve cellular interaction with the implanted biomaterial (7-8,22).The anodization process explored in this current study presented this morphology both in the laboratory titanium disc samples and commercial implants treated by this methodology. Another characteristic of great interest for biomedical implants is the changes in terms of wettability as anodization can alter both hydrophilic and hydrophobic surfaces (4, 24).
Contaminations by surface treatments are demonstrated in several studies (10, 25), some of the elements found are considered cytotoxic (11) and a more critical evaluation in its use in biomedical implantology is needed. The proposed surface treatment did not reveal any surface contamination suggesting anodization is a suitable surface treatment method for biomedical implants.
Particle cytotoxicity has been reported by a large number of researchers, from the use of nanoparticles and by the corrosion of biomaterials (26-27). Biomaterials contaminated with elements such as aluminum, chromium, nickel, among others are still widely used in the current biomedical implants. Its gradual release due corrosion or contamination directly into the bone tissue is a concern. The human body has difficulty removing these elements from the circulation and they could bioaccumulate inside the body (28). The anodizing process together with a pure titanium alloy yields a biomaterial surface that is totally clean and free of cytotoxic elements.
Surface treatment involves processes using liquids or solids such as acid leaching or particle blasting (1,12,25, 29). Treatment processes involving liquids react more critically on the material making it difficult to contaminate or impregnate other chemical elements. While processes using solid particles normally using materials other than titanium, may increase the risk of surface contamination by residues of the sandblasting particles or introducing additional impurities existed in blasting material (12,25). The anodization performed in this study proved to be a desirable process that produces a product free of residues or surface contaminations.
In addition, anodization allows the functionalization for the delivery of drugs or nanoparticles inside the nanotubes (TNTs). Zhang et al. and Hua et al. (24,30) reported the incorporation of silver or copper nanoparticles proving great antibacterial potential and biocompatibility, respectively. However, these particles in a high degree of release could generate bioaccumulation in the human body because they are not easily excluded (31). On the other hand, other studies (20-22) show the functionalization for the delivery of drugs (e.g. proteins, growth factors, and peptides) that are easily degraded by the recipient demonstrating high potential of antibacterial properties and increased speed of bone healing.
The limitations of this study do not allow to extrapolate conclusions with regard to the resultant surface is cytotoxic or not. Nevertheless, it allows a physicochemical demonstration of the present or absence of elemental contaminations on the surface of various implants. The results also indicate that anodization is a promising pro-

cess that can yield surface contaminant free biomedical implants. Future research should consider cell culture toxicity studies and in-depth bioaccumulation analyzes to prove the actual efficacy of a given surface treatment.

CONCLUSIONS

The process by electrochemical anodization allows the formation of a surface with nanomorphology and potential application for functionalization. Its surface does not appear to have any elemental contamination when pure titanium is used following the practice in implant manufacturing processes. The apparent lack of elemental contamination of the anodized surface would suggest non-cytoxicity of this type of implants although further investigation is needed. It is highly promising for its use in biomedical applications, and affords opportunity for developing implants including oral implants with drug delivery systems.

Declaration of conflicting interests
The Authors declares that there is no conflict of interest.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Authorship
Marcel Ferreira Kunrath: Conception, acquisition, interpretation, draft, revision and agreement to publish the work. Nilton Penha: Acquisition, interpretation, revision and agreement to publish the work. Jack C. Ng: Interpretation, draft, revision and agreement to publish the work.

REFERENCES

1. Civantos A, Martinez-Campos E, Ramos V, et al. Titanium coatings and surface modifications: toward clinically useful bioactive implants. ACS Biomater Sci Eng 2017; 3: 1245-61.
2. Hotchkiss KM, Reddy GB, Hyzy SL, et al. Titanium surface characteristics, including topography and wettability, alter macrophage activation. Acta biomater 2016; 31: 425-34.
3. Jimbo R, Wennerberg A, Albrektsson, T. Experimental and clinical knowledge of surface micro-topography. In Implant Surfaces and their Biological and Clinical Impact 2015: Springer, Berlin, Heidelberg; 2015 pp.13-20.
4. Awad NK, Edwards SL, Morsi YS. A review of TiO2 NTs on Ti metal: Electrochemical synthesis, functionalization and potential use as bone implants. Mater Sci Eng C 2017; 76: 1401-12.
5. Kunrath MF, Leal BF, Hubler R, et al. Antibacterial potential associated with drug-delivery built TiO 2 nanotubes in biomedical implants. AMB Express 2019 9: 51.
6. Kunrath MF, Hubler R, Shinkai RS, Teixeira ER. Application of TiO2 nanotubes as a drug delivery system for biomedical implants: a critical overview. ChemistrySelect 2018; 3: 11180-9.
7. Park J, Bauer S, Schlegel KA, et al. TiO2 Nanotube Surfaces: 15 nm - An Optimal Length Scale of Surface Topography for Cell Adhesion and Differentiation. Small 2009; 5:666-71.
8. Yun KD, Yang Y, Lim HP, et al. Effect of nanotubular-micro-roughened titanium surface on cell response in vitro and osseointegration in vivo. Mater Sci Eng C 2010; 30:27–33.
9. Mazare A, Totea G, Burnei C, et al. Corrosion, antibacterial activity and haemocompatibility of TiO2 nanotubes as a function of their annealing temperature. Corrosion Sci 2016; 103: 215-22.
10. Penha N, Groisman S, Ng J, et al. Physical-chemical analyses of contaminations and internal holes in dental implants of pure commercial titanium. J Osseointegration 2018; 10: 57-63.
11. Kopp B, Zalko D, Audebert M. Genotoxicity of 11 heavy metals detected as food contaminants in two human cell lines. Environmental and molecular mutagenesis 2018; 59: 202-10.
12. Marenzi G, Impero F, Scherillo F, et al. Effect of Different Surface Treatments on Titanium Dental Implant Micro-Morphology. Materials 2019; 12: 733.
13. Sul YT, Johansson CB, Röser K, Albrektsson T. Qualitative and quantitative observations of bone tissue reactions to anodised implants. Biomaterials 2002;23:1809–17.
14. Ionita D, Bajenaru-Georgescu D, Totea G, et al. Activity of vancomycin release from bioinspired coatings of hydroxyapatite or TiO nanotube. Int J Pharm 2017; 517:296–02.
15. Nemati S, Hadjizadeh A. Gentamicin-eluting titanium dioxide nanotubes grown on the ultrafine-grained titanium. AAPS Pharm Sci Tech 2017;18:2180–7.
16. Roguska A, Belzarz A, Zalewska J, et al. Metal TiO2 nanotube layers for the treatment of dental implant infections. ACS Appl Mater Interfaces 2018; 10:17089–99.
17. Yao S, Feng X, Lu J, et al. Antibacterial activity and inflammation inhibition of ZnO nanoparticles embedded TiO2 nanotubes. Nanotechnology 2018; 29:244003.
18. Pawlik A, Jarosz M, Syrek K., Sulka GD. Co-delivery of ibuprofen and gentamicin from nanoporous anodic titanium dioxide layers. Colloids Surf. B: Biointerfaces 2017; 152: 95–102.
19. Wang T, Weng Z, Liu X, et al. Controlled release and biocompatibility of polymer/titania nanotube array system on titanium implants. Bioactive Mater. 2017; 2: 44–50.
20. Somsanith N, Kim YK, Jang YS, et al. Enhancing of Osseointegration with Propolis-Loaded TiO2 Nanotubes in Rat Mandible for Dental Implants. Mater 2018; 11, 61.
21. Zhang X, Zhang Z, Shen G, Zhao J. Enhanced osteogenic activity and anti-inflammatory properties of Lenti-BMP-2-loaded TiO2 nanotube layers fabricated by lyophilization following trehalose addition. Int. J. Nanomedicine 2016; 11, 429–39.
22. Shim IK, Chung HJ, Jung MR, et al. Biofunctional porous anodized titanium implants for enhanced bone regeneration. J Biomed. Mater. Res. A 2014; 102, 3639–48.
23. Li T, Wang N, Chen S, et al. Antibacterial activity and cytocompatibility of an implant coating consisting of TiO2 nanotubes combined with a GL13K antimicrobial peptide. Int. J. Nanomedicine 2017;12, 2995–07.
24. Zhang L, Zhang L, Yang Y, et al. Inhibitory effect of super-hydrophobicity on silver release and antibacterial properties of super-hydrophobic Ag/TiO2 nanotubes. J Biomed Mater Res B 2016; 104, 1004-12.
25. Guler B, Uraz A, Çetiner D. The chemical surface evaluation of black and white porous titanium granules and different commercial dental implants with energy-dispersive x-ray spectroscopy analysis. Clin implant dent relat res 2019; 21:352-9.
26. Manam NS, Harun WSW, Shri DNA, et al. Study of corrosion in biocompatible metals for implants: A review. J Alloys Compounds 2017; 701: 698-15.
27. Asri RIM, Harun WSW, Samykano M, et al. Corrosion and surface modification on biocompatible metals: A review. Mater Sci Eng C 2017; 77: 1261-74.
28. Lanocha-Arendarczyk N, Kosik-Bogacka DI, Kalisinska E, et al. Influence of environmental factors and relationships between vanadium, chromium, and calcium in human bone. BioMed res int. 2016; 2016, Article ID 8340425.
29. Santos Tavares H, Silveira Faeda R, Carlos Guastaldi A, Pozzi Semeghini Guastaldi F, Oliveira NT, Marcantônio E Jr. E Jr. SEM-EDS and biomechanical evaluation of implants with different surface treatments: an initial study. J Osseointegr 2009;1(1):15-21.
30. Hua Z, Dai Z, Bai X, et al. Copper nanoparticles sensitized TiO2 nanotube arrays electrode with enhanced photoelectrocatalytic activity for diclofenac degradation. Chem Eng J 2016; 283: 514-23.
31. Charabi Y, Choudri BS, Ahmed M. Ecological and human health risk assessment. Water Environment Res 2018; 90: 1777-91.