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BIOSURFACE PROCESSING WITH ROLE IN IMPROVING THE OSSEOINTEGRATION OF THE ORAL IMPLANT

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ABSTRACT

Introduction Obtaining and maintaining tissue integration is ensured by an implant biosurface design with a role in reducing the effect of shear forces at the interface and which can stimulate osteogenesis or create conditions to facilitate post-implant healing.
The role of the implant surface roughness in stimulating and improving bone growth to the implant surface, especially in low bone density structures has been frequently emphasized in studies described in the literature.
Methodology The present study reveals the concerns of the authors regarding the influence of surface processing of samples of titanium bioalloys. The modification of the biosurface parameters (micro-roughness) was evaluated, as well as their influence on the adhesion of osteoblasts on experimentally processed surfaces and the cell proliferation capacity by evaluating biocompatibility in vitro. The experiments used micro-topographic analysis of the sample surface by atomic force microscopy (AFM) which provided useful information on the roughness profile and parameter values that characterize the profile groups of roughness, 3D and scanning electron microscopy analyzes (SEM and EDS) that highlighted changes in the morphology of the experimentally processed biosurface.
Results Some of the research results were presented, which aimed to establish the optimal way to modify the biosurface area through different processes for its processing.
Conclusions The results of this experimental study together with those previously presented in other scientific papers demonstrate the abilities of the Ti10Zr bioalloy and confirm the efficiency of surface

KEYWORDS

Bioalloy, Implant, Biosurface, Anodic Oxidation, Cell Proliferation.

1. INTRODUCTION

Bone healing in the post-implantation period involves the onset of cellular and extracellular biological processes at the bone-implant interface, completed with the formation of new bone [1].
The first reactions result in the formation of an interface, in which the tissue is invaded by the liquid phase consisting mainly of blood, a multi-protein solution [2,3]. The biological processes at the bone-implant interface are controlled by growth and differentiation factors released by blood cells. The platelets undergo morphological and biochemical changes in response to the interaction with the external surface, and intracellular biochemical changes lead to the induction of phosphotyrosine, the increase of intracellular calcium and the hydrolysis of phospholipids. The fibrin matrix formed acts as a

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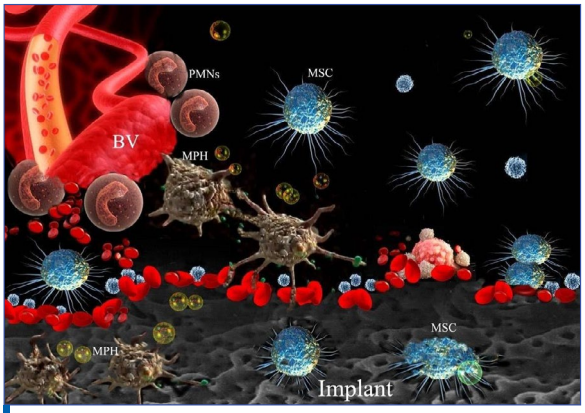

Figure 1. Bone-implant interface; BV - blood vessel, MSC - mesenchymal cell, PMN - leukocytes, MSP - macrophages [14].

skeleton that ensures the migration of osteogenic cells and ultimately produces the differentiation of these cells in the healing area. Osteogenic cells form the osteoid tissues of the trabecular bone, which eventually reshape into lamellar bone in direct contact on the implant surface [4-13]. The newly formed bone develops towards the surface of the implant, and the newly formed network of bone trabeculae ensures the biological fixation of the implant. Fig. 1 suggestively illustrates a possible line of interaction between cells and the implant surface during the initial healing phase. Obtaining the osseointegration of the implant is conditioned by the presence of a biocompatible material, an osteoinductive surface and a design that optimally projects the implant surface [15].

In the case of the titanium implant, when the connection with the bone tissue elements is established at the molecular level, the success of osseointegration is ensured by the tissue compatibility, a feature that influences the healing, restoration and remodelling process in the tissues after implantation. The shortening of the post-implantation healing period and implicitly of the osseointegration duration was proved by improving the cellular interaction at the interface between the organism and the biomaterial (Ratner and Porter, 1996) by changes in the morphology of the implant surface. Nanometrically modified metal surfaces have been shown to promote cell adhesion and proliferation, as increasing the area of the implant's biosurface actually increases the available space and thus increases the number of cells that will adhere to the surface. Obtaining porous surfaces with a certain pore size controls the processes of adhesion and bone apposition. Micropores allow the adhesion of proteins, and macropores with a diameter greater than 100-150 micrometers facilitate blood supply (These Goalard). In vitro research on titanium implants with different surface micro-topographies has demonstrated the differentiation of bone and mineralization cells in close dependence with their roughness. In general, rough surfaces favor osseointegration through the attachment of

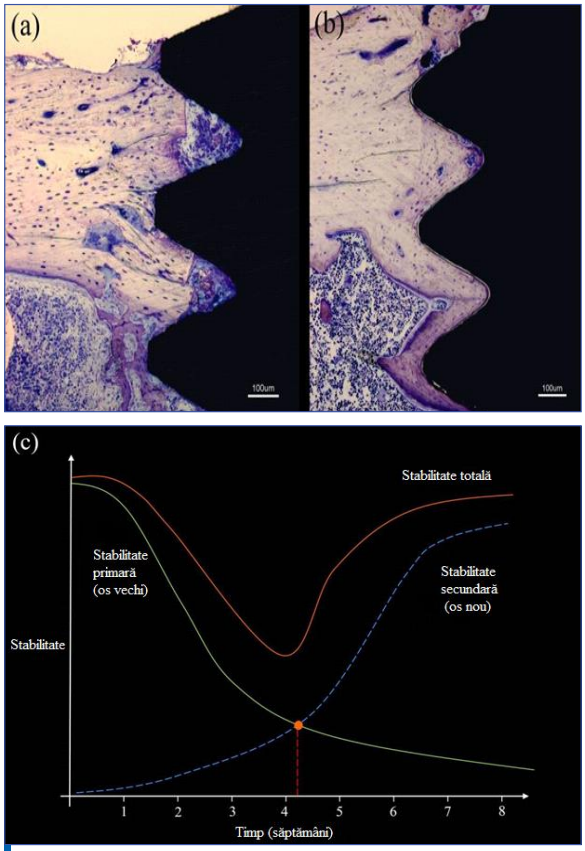

Figure 2. High primary (mechanical) stability of the implant achieved by the bone bed preparation technique (a), Secondary (biological) stability obtained by the formation of new bone (b), Duration of the healing phase (c) [15,17].

osteoblasts and subsequent proliferation also by the size of the implant-bone contact area, with a positive influence on the primary stability of the implant.

At the same time, the surface chemistry (Kilpadi and Lemons, 1994) as well as the hydrophilic/hydrophobic balance are factors that influence the processes at the implant-tissue interface. In addition to the fact that the wettability of the material which is also a predictable indicator of cytocompatibility, the hydrophilic character influences both the cell attachment and the degree of cell proliferation on the surface of the material.

The successful modification of biosurface parameters was achieved by the surface treatment with hydroxyapatite (HA) performed by various methods such as: plasma spray deposition, high viscosity flame spray, glazing, pulsed laser deposition, pulsed electron deposition, magneto-sputtering deposition, electrophoretic deposition, chemical vapor deposition, physical vapor deposition, HA blast coating, sol-gel deposition, melt immersion, chemical deposition of biological HA from solutions similar to blood plasma on surfaces activated with H_2O_2 or bases, chemical deposition of biological HA from solutions similar to blood plasma in the presence of biovitroceramics, etc. The results of the Romanian research regarding the surface treatment with hydroxyapatite [D. Mariş, M. Mariş, M. Mariş] [16] showed that obtaining superficial microretentions

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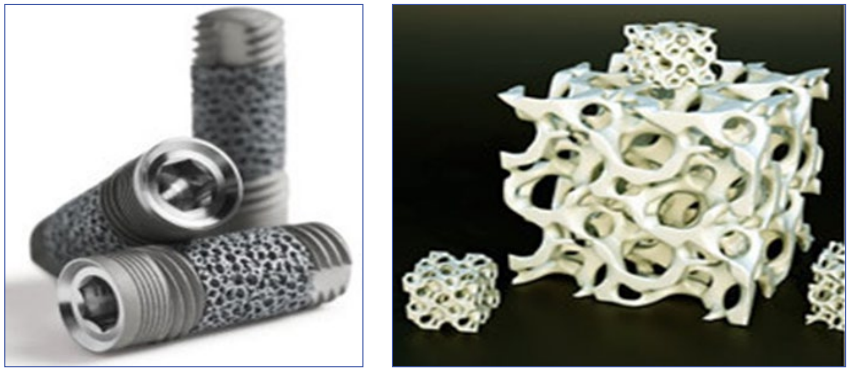


Figure 3. Built-in osteo implant (Trabecular Metal) [15,18].

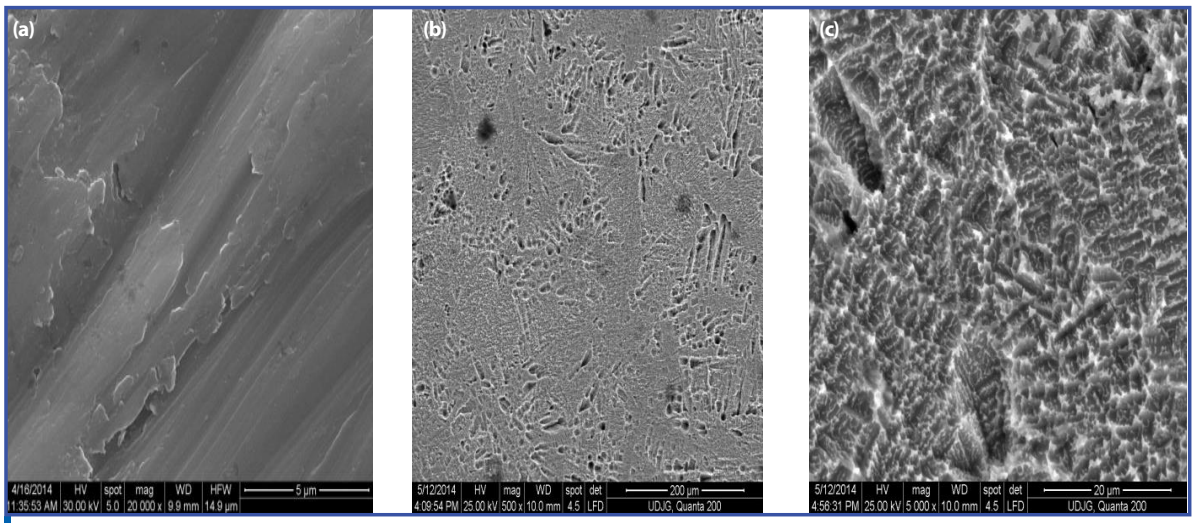


Figure 4. Microscopic aspects of the surface of the Ti10Zr samples experimentally processed through: casting + machining + grinding (a), acid corrosion (b), anodic oxidation (c).

contributes to the osseointegration of the implant, "a better bone apposition was observed around the implant, especially in the early period of healing".

Recent research on the peculiarities of in vivo histointegration of surface-treated titanium dental implants has comparatively evaluated the process of osseointegration of surface-treated titanium implants by mechanical methods and by chemical coating with hydroxyapatite.

The treatment of the implant surface with acids, plasma or other methods that aimed to increase the roughness to improve the parameters of the implant biosurface, but also differentiated treatment, are elements of progress in implant design, which led to faster healing and better stability of the implant.

The modern design introduced by the "Trabecular Metal" implant (Fig. 3) conceptually revolutionized the theory of osseointegration and introduced the notion of osseoincorporation (bone tissue growth also in the implant structure). Considered the newest discovery in the field of dental implantology, it is the only implant with a three-dimensional structure (3D) that mimics bone cell architecture (80% porosity) and systematic nanotextured topography of superficial areas. The trabecular structure of the implant causes the bone to form inside it, resulting in a common body between the implant and the human bone. The implants have a treated surface of SLA type (Sandblasting with Large grit followed by Acid etching), chemically modified and moderately rough, which increases the bone-implant contact surface.

The paper presents part of the research results on the characteristics of experimentally processed surfaces with a role in intensifying biological processes at the biomaterial-tissue interface. It was considered to establish the optimal way to modify the biosurface area of the oral implant, between mechanical, chemical and electrochemical processing procedures. The studied experimentally processed surfaces were of Ti10Zr bioalloy.

2. MATERIALS AND METHOD

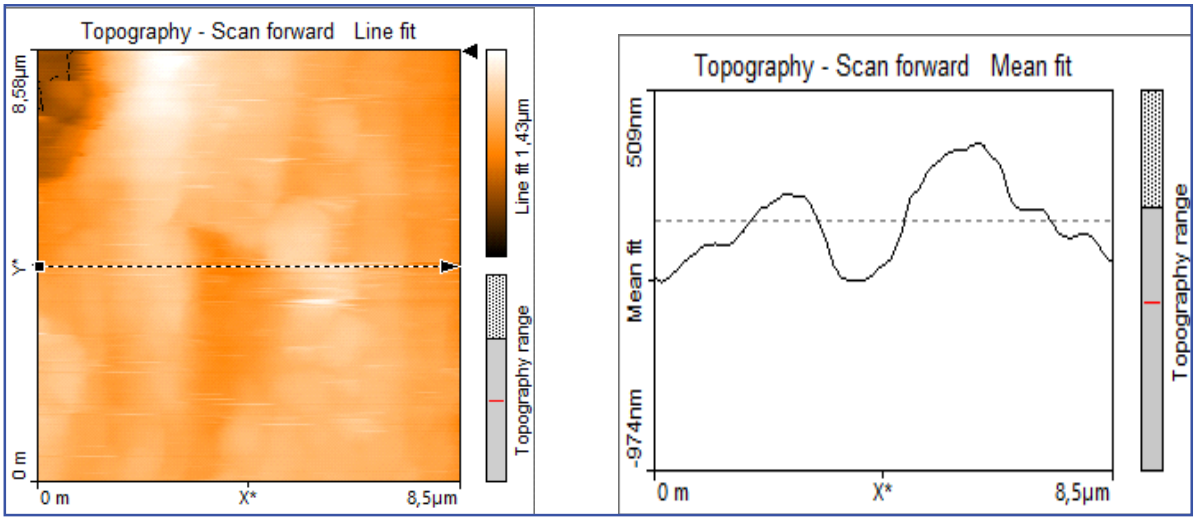
In the experiments, samples from the experimental bioalloy with a titanium base were used (Ti10Zr / Patent no. 132079 / 28.06.2019). The samples were taken from the moulded semi-finished product subsequently subjected to processing (casting + rectifying), mechanical processing (rectifying + grinding) or surface treatments by acid attack and anodic oxidation (previously published results [19]).

The analysis of the sample surface was performed by atomic force microscopy (AFM / EasyScan2 Model) and scanning electron microscopy (SEM), which provided useful information on the roughness profile and the values of the parameters that characterize the roughness profile groups, and the investigation

Original Articles

a. Samples taken from cast semi-finished products and subsequently subjected to mechanical processing (rectifying)

a.1. 2D images



a.2. 3D images

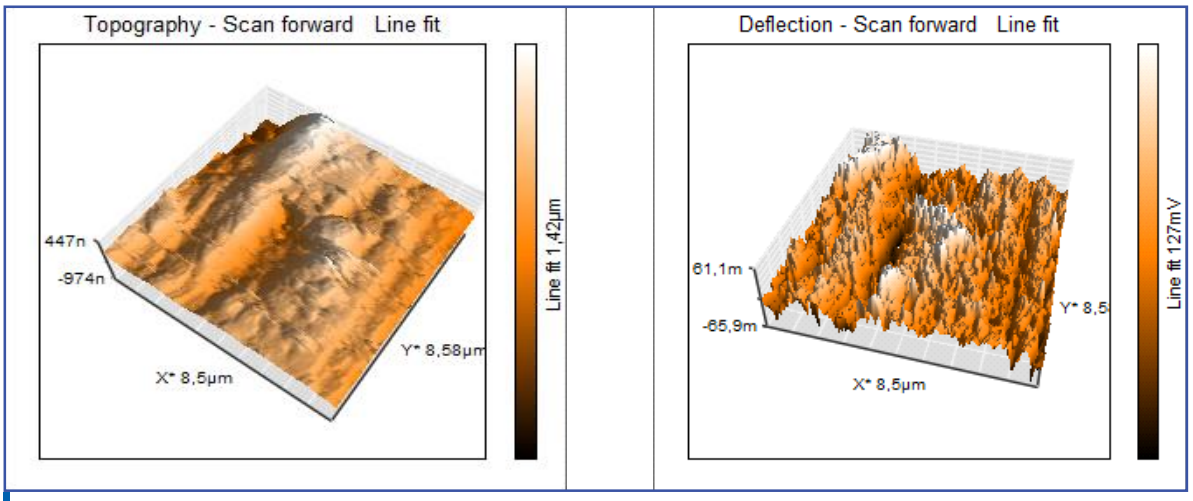


Figure 5. Parameters that characterize the roughness profile.
Ra = 125,62nm, Rq = 147,75nm, Ry = 525,97nm, Rp = 266,18nm, Rv = - 259,79nm, Rm = - 3,628fm.

and evaluation of interactions at the interface level was performed by in vitro analysis by exposing G292 osteoblasts to these surfaces.

3. RESULTS

The analysis of the experimental samples by scanning electron microscopy (SEM / Fig. 4) and atomic force microscopy (AFM / Fig. 5) highlighted the changes in the morphology of the experimentally processed biosurface and their influence on the biological processes at the interface, demonstrated by in the vitro experiments (Fig. 6).

Remarks:
The anodic oxidation method allows the development of an oxide layer on the surface of the material with a role in improving the adhesion and fixation properties. The Ti10Zr alloy samples thus processed provide a special surface configuration, as shown in the electron scanning microscopy images (Fig. 4c). The oxide film is a basis for the formation of the osteoinductive matrix [19]. The following are the microscopic aspects of the sample surface and the parameters that characterize the roughness profile, electron microscopy analysis (AFM), 2D and 3D images of the experimentally processed sample surface.

a. Samples taken from cast semi-finished products and subsequently subjected to mechanical processing (rectifying)

b. Samples subjected to mechanical processing (rectifying + grinding)

The micro-topographic analysis of the sample surface through atomic force microscopy (AFM) provided useful information on the roughness profile and the values of the parameters that characterize the roughness profile groups. The roughness corresponds in value to the surfaces

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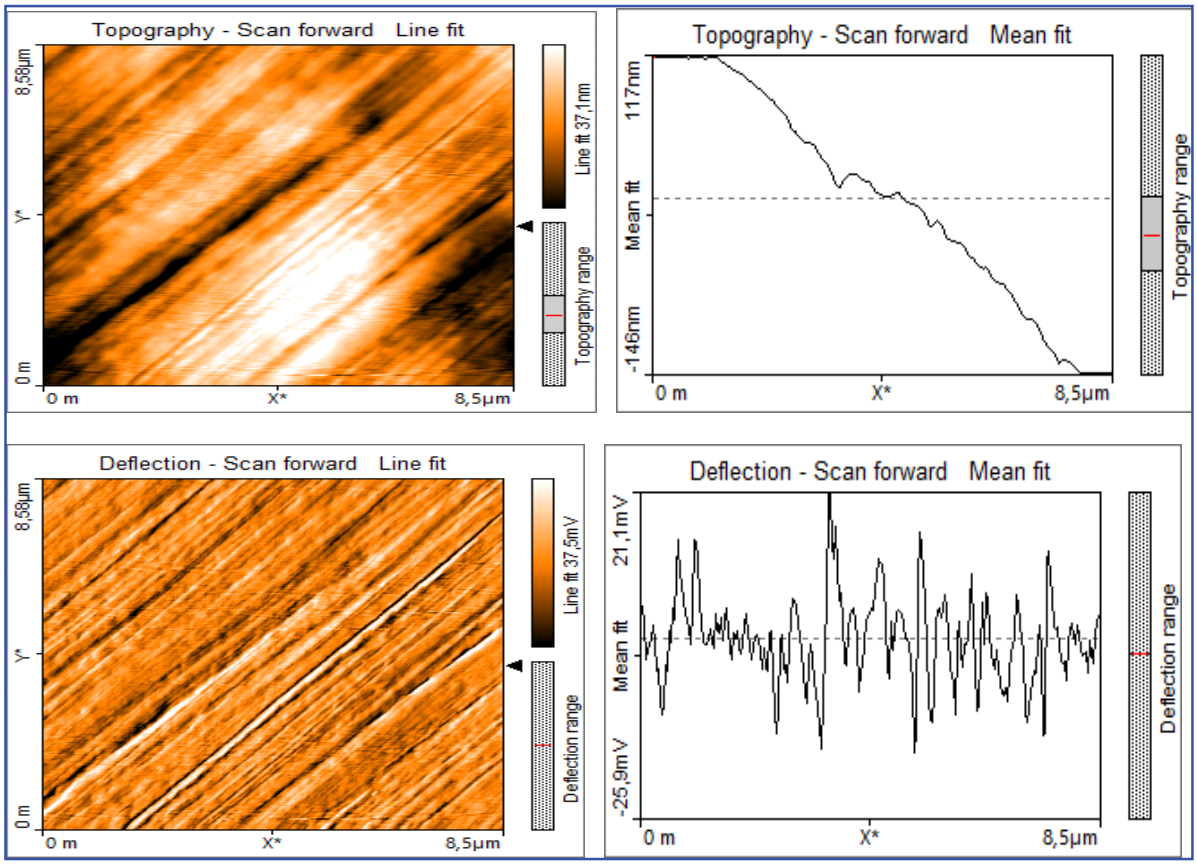
b. Samples subjected to mechanical processing (rectifying + grinding)

b.1. 2D images



b.2. 3D images

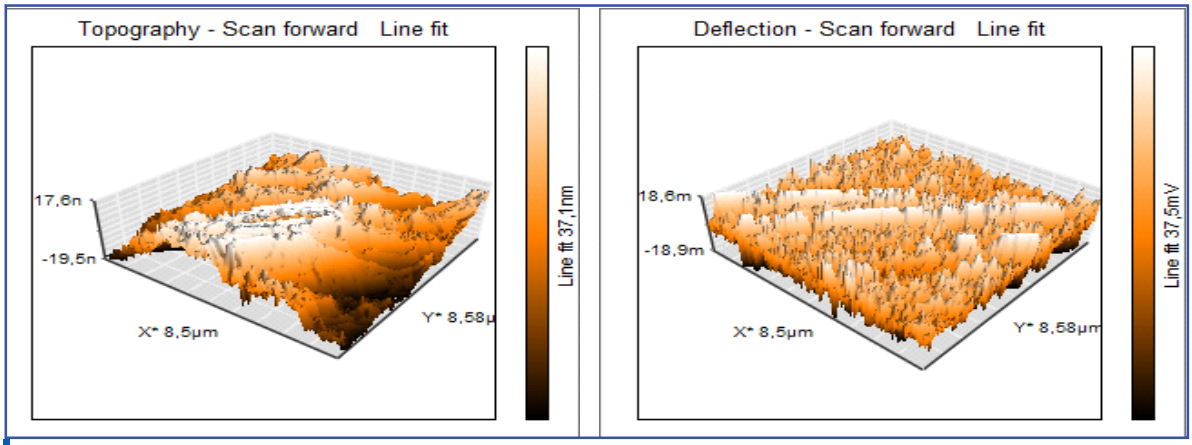


Figure 6. Parameters that characterize the roughness profile
Ra = 7,9282 nm, Rq = 9,1452nm, Ry = 43,537nm, Rp = 16,564nm, Rv = - 26,973nm, Rm = - 3,4725fm.

with very fine processing (Fig. 6). There are isolated high-roughness tips with a rounded shape, which is a characteristic of materials processing. The investigation and evaluation of the interactions between osteoblasts and the surfaces of the TiZr samples was performed by exposing the osteoblasts to these surfaces. G292 osteoblasts were seeded in 6-well plates at a density of 5 x 104 cells / cm^2 in the presence of the Ti10Zr samples with differently processed surfaces; In parallel, cells were seeded also directly on the surface of the culture vessels in the absence of any material (control). After 24 and 48 hours of incubation, a medium was harvested from each well to determine the degree of LDH release and the fluorescent labelling of the cytoskeletal actin filaments and intracellular glutathione was performed. [20]. After examining the architecture of the actin filaments under the fluorescence microscope after 24 and 48 hours of culture on the surface of the Ti10Zr (Fig. 7) it was observed that the cells grew in monolayer and showed an osteoblast-like phenotype, with no major differences regarding the organization of F-actin between these cells and the control ones. There was a good adhesion of osteoblasts on these surfaces, the cells having a well-organized actin cytoskeleton, with cytoplasmic

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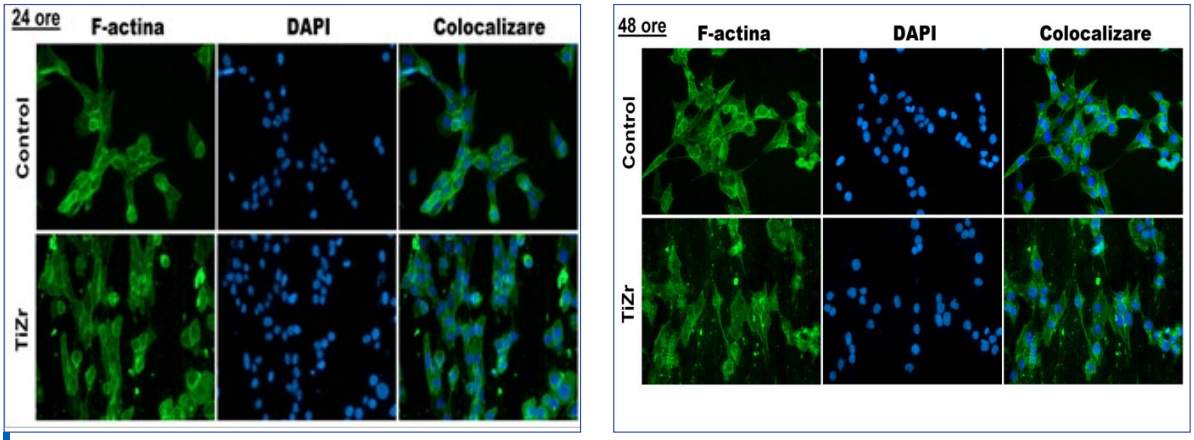

Figure 7. Highlighting the actin cytoskeleton by fluorescent labelling of F-actin with phalloidin-FITC (DAPI nucleus counter colouring) in osteoblasts grown for 24 and 48 hours on the surface of the culture vessel or Ti10Zr alloy [15, 20].

extensions that interconnect neighbouring cells, which are arranged at cell densities comparable to those of the control. What is worth noting is the orientation of the adhered cells in the direction of grinding of the experimental samples, which suggests that the processing changed the properties of these surfaces, and implicitly decisively influenced the orientation and adhesion of the cells. The evaluation of the glutathione levels in the cells that define their proliferative capacity and quantification of GSH-CMF fluorescence in adherent cells on the sample surface [15,20] showed that the nuclear and cytoplasmic GSH levels are similar to control after 24 hours, increasing by 12%, respectively 17%, after 48 hours. This increased level proves that the surface of the material stimulates the cell proliferation due to their properties.

4. CONCLUSIONS

The research highlights the possibilities of processing the biosurface of the implant playing a role in improving the biological processes at the implant-tissue interface in the immediate post-implantation period. Part of the research results on the influence of surface processing of samples of experimental titanium bioalloys on changes in morphology and biosurface area by mechanical processing is presented. The modification of the biosurface area parameters was evaluated by measuring the parameters that characterize the roughness profile groups, as well as their influence on the adhesion of osteoblasts and the cell proliferation capacity on the experimentally processed surfaces.

The results of this experimental study together with those previously presented in other scientific papers demonstrate the abilities of the Ti10Zr bioalloy and confirm the efficiency of surface microtopography modification processes to improve implant osseointegration by intensifying cellular processes at the bone-implant interface in the post-implantation period.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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None.

AUTHOR CONTRIBUTIONS

VGV: participated in the elaboration, writing and translation of the paper and contributed to the introductory part (synthesis of specialized information on the topic), to establishing the experimental conditions, interpreting the results and formulating the research conclusions. EV: contributed as follows: characterization of the materials researched and the interpretation of the results obtained in the investigation by advanced methods (SEM Microscopy, EDS Analysis). VS: participated in the writing and translation of the paper and contributed to structuring the bibliographical references. LTC: participated in the research of the documentary sources (bibliographical references), in the structuring of the research conditions and methodology and in the elaboration of the abstract.

REFERENCES

[1] P.I. Brånemark Osseointegration and its Experimental Studies, J.Prosthet. Dent., 1983 1983 Sep;50(3):399-410. doi: 10.1016/s0022-3913(83)80101-2. Osseointegration and its experimental background - PubMed (nih.gov)

[2] http://www.scritub.com/medicina/Osteointegrarea743186.php

[3] R. G. Craig, Restorative dental materials, Ed. All Educational Bucharest 2001, p.180, Craig's Restorative Dental Materials - 13th Edition (elsevier.com)

[4] P.I. Bränemark, Introduction to osseointegration,Tissue Integrated Prostheses: Osseointegration in Clinical Dentistry. Chicago: Quintessence, 1995, p. 11-76, www.quintpub.com, Tissue-Integrated Prostheses: Osseointegration in Clinical Dentistry: 9780867151299: Medicine & Health Science Books @ Amazon.com

[5] A. Jokstad,Osseointegration and Dental Implants, 2008 John

Wiley & Sons, Inc. ISBN: 978-0-813-81341-7, Osseointegration and Dental Implants | Wiley Online Books

[6] I. Palada, A. M. Smarandache, M. Sabău, The Concept of osseointegration in implantology, Acta Medica Transilvanica, vol II, nr. 2, 2012, p. 113, conceptul de osteointegrare in implantologie - Acta Medica ... (yumpu.com)

[7] http://www.unitbv.ro/Portals/31/Sustineri%20de%20doctorat/Rezumate/Pitulice Camelia_v2.pdf

[8] A. Popovici, Bone integration pf oral implants, PhD thesis, Bucharest, 2008

[9] T.Albrektsson, Bone tissue response in: Bränemark PI, Zarb G, Albrektsson T., eds. Tissue Integrated prostheses: Osseointegration in Clinical Dentistry, Chicago, 1985, p. 129-143 Tissue-Integrated Prostheses: Osseointegration in Clinical Dentistry (quintpub.com)

[10] A. F. Mavrogenis, R. Dimitriou J. Parvizi, G.C. Babis, Biology of Implant Osseointegration, J. Musculoskelet Neuronal Interact 2009; 9 (2):61-71, Biology of implant osseointegration - PubMed (nih.gov)

[11] R. Mustafa, Osseointegration and Bioscience of Implant Surface- Current Concepts at Bone- Implant Interface, Implant Dentistry, A Rapidly Evolving Practice ,www.intechopen.com

[12] S. Anil, P.S. Anand, H. Aghamdi, J.A. Jansen., Dental Implant Surface Enhancement and Osseointegration, www.intechopen.com.

[13] W.J. O'Brien, Dental Materials and Their Selection, 3rd Ed., 2002, Chapter 23, Quintessence Publishing Co, Inc 4350 Chandler Drive Hanover Park, IL 60133 www.quintpub.com

[14]A. Ballo, Implant Dentistry Research Guide Basic, Translational and Clin. Research, 2012, (PDF) Implant Dentistry Research Guide: Basic, Translational and Clinical Research (researchgate.net)

[15] V.G. Vasilescu, Contributions to the study of biocompatible metallic materials for oral implantology, PhD Thesis, UMF Bucuresti, 2016, Rezumat_ Teza _Doctorat (umfcd.ro)

[16] D. Mariş, M. Mariş, M. Mariş, Peculiaities of the in vivo histointegration of surface-treated titanium implants, Romanian Journal of Dentistry, vol. IV, no. 3, 2009

[17] R. M. Palmer, L.C. Howe, P. J. Palmer, Implants in Clinical Dentistry, Second Edition 2002, Implants in Clinical Dentistry - 2nd Edition - Richard M. Palmer - Le (routledge.com)

[18] M. Collins, J. Bassett, Hai Bo Wen, Chris Gervais, Matt Lomicka, Savvas Papanicolaou, Trabecular Metal Dental Implants:Overview of design and developmental research, 2012 by Zimmer Dental Inc., [PDF] Trabecular Metal ™ Dental Implants: Overview of design and developmental research | Semantic Scholar

[19] Elisabeta Vasilescu1, Vlad Gabriel Vasilescu2, Ion Patrascu2, Controlled Changing of Implantable Bioinert Materials Biosurface, Romanian Journal of Materials, 2016, 46 (1). pag.11-16, Vlad Gabriel Vasilescu - Google Academic ,

[20] Vlad Gabriel Vasilescu, Miruna Silvia Stan, Ion Patrascu, Anca Dinischiotu, Elisabeta Vasilescu, In Vitro Testing of Materials Biocompatibility with Controled Chemical Composition, Romanian Journal of Materials, 2015, 45 (4), pag. 315-323 , Vlad Gabriel Vasilescu - Google Academic

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Assistant Professor, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy Bucharest, Department of Prosthesis Technology and Dental Materials. In 2016, I was awarded my PhD in Dentistry with the thesis entitled "Contributions to the study of biocompatible metal materials for oral implantology". Areas of interest in the research activity: obtaining and characterizing dental materials and highly biocompatible materials, characterizing implantable systems in relation to the biocompatibility of materials and surface microtopography. Disseminating research results involves communications at prestigious scientific events and publications in specialized journals, awards and distinctions: Gold Medal for "High biocompatibility alloy for dental implants", Diploma of Excellence for the works "Electron Microscopy Studies of Depositing Metallic Silver with Antibacterial Role on the TiZr Dental Implant Surface".

Original Articles

Questions

1. Improving the osseointegration of the implant is possible by:

❑a. the modification of the microtopography that determines the increase of the biosurface area;
❑b. use of implants with smooth (unprocessed) surfaces;
❑c. early implant loading;
❑d. the use of materials with high fatigue resistance.

2. Changes in biosurface morphology by anodic oxidation have the following effect:

❑a. increase of the oxide layer on the implant surface;
❑b. improve osteoinductive properties;
❑c. decrease biological processes at the tissue-implant interface;
❑d. decreased cell adhesion.

3. Maintaining tissue integration is improved:

❑a.by an implant biosurface design that increases the effect of shear forces;
❑b. by factors that diminish the primary bone-implant stability;
❑c. by a design of the implant biosurface that reduces the effect of shear forces;
❑d. in structures with low bone density.

4. The role of the microroughness of the implant surface is:

❑a. to inhibit the growth of bone tissue to the surface of the implant;
❑b. to improve protein adsorption in the cellular interaction at the interface between tissue and biomaterial ;
❑c. to reduce the bone-implant contact surface;
❑d. to prevent the interfacial reaction in the post-implantation period.