IN MEMORIAM Prof. Dr. Ing. PETRU BALTĂ

MODIFICAREA CONTROLATĂ A BIOSUPRAFEȚEI MATERIALELOR BIOINERTE IMPLANTABILE
CONTROLLED CHANGING OF IMPLANTABLE BIOINERT MATERIALS BIOSURFACE

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The quality of the biomaterial in implantology is assessed by its ability to allow rapid osseointegration. Tissues react differently to implant depending on the type of biomaterial while the nature of reactions at implant - tissue interface makes a clear distinction between bioinert, biotolerated, bioactive materials. Achieving tissue biointegration requires the presence of a biocompatible material, and also an optimally designed biosurface of the implant. Increasing the biosurface area , possible through various processing methods (mechanical, chemical, electrochemical, etc.), favors the implant osseointegration by the size of the implant - bone contact zone that influence the process of adherence, proliferation and cell growth. This study analyzes the results of the research on the conditions for modifying the surface microtopography of a new material, Ti10Zr bio alloy, by machining, anodic oxidation and acid corrosion and the cell response to surface modification.

X-ray diffraction indicated the presence of oxides TiO_2 (anatase) and scanning electron microscopy analysis of experimental surfaces processed revealed a special configuration of the surface oxidized , particularly from those obtained by anodizing.

Calitatea biomaterialului în implantologie este apreciată prin capacitatea sa de a permite o osteointegrare rapidă. Țesuturile reacționează la implantare în mod diferit, în funcție de tipul de biomaterial, iar natura reacțiilor de la interfața implant - țesut face o distincție clară între materialele bioinerte, biotolerate, bioactive.

Obținerea biointegrării tisulare necesită prezența unui material biocompatibil, dar şi o biosuprafață optim proiectată a implantului. Creşterea ariei biosuprafeței, posibilă prin diferite metode de prelucrare (mecanică, chimică, electrochimică, etc) favorizează osteointegrarea implantului prin mărimea zonei de contact implant – os, care influențează procesele de aderare, proliferarea şi creşterea celulară. Studiul de față relevă rezultatele cercetărilor privind condițiile de modificare a microtopografiei suprafeței unui nou material, bioaliajul Ti10Zr, prin prelucrare mecanică, coroziune acidă şi oxidarea anodică şi răspunsul celular la modificarea suprafeței. Difracția de raze X a indicat prezența oxizilor TiO_2 (anatase) iar analiza de microscopie electronică cu baleiaj a suprafețelor experimental procesate a evidențiat o configurație specială a suprafeței oxidate, în mod deosebit la cele obținute prin anodizare.

Keywords: *bioalloy, citotoxicitaty, biocompatibility, anodic oxidation, biosurface, cell proliferation, cell adhesion.*

1. Introduction

Biomaterials intended for implant should be able to combine intrinsic biocompatibility (biomaterial chemically and biologically compatible with the host tissue) with extrinsic or functional biocompatibility (biomaterial meets all the physical and mechanical properties required by the type of application and the optimum transmission capacity of the implant-tissue interface strains) [1 - 3].

Branemarck (1982) underlined the importance of biocompatibility of the materials used in oral implantology relative to bone structures and introduced the term of osseointegration.

The success of the whole process of implantation, according to Branemark, depends on "how closely and tightly the bone grows around the implant" [4 - 8].

In 1987 Meffert redefines and divides the term of osteointegration into ,,adaptive osteointegration", as defined by the existence of bone tissue in rough contact with the implant surface or lack of interface soft tissue and ,,biointegration", as defined by the existence of biomechanical adhesions between implant and bone tissue.

Johanson (1986), Albrektsson and Jacobson (1987) show that osteointegration means the presence of the regenerated bone in the next vicinity of the metal and is a direct and sustainable link between the live and reshuffled bone and at least 90% of the implant surface on its transcortical section.

Tissues react to implantation in a different way, depending on the type of biomaterial used, and the mechanism of tissue attachment depends on its response to the implant surface, namely the properties of the material presence [9]. The nature of the reactions at the implant - tissue interface makes a clear distinction between bioinert, biotolerated, bioactive materials.

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Among the classes of materials used (metal, ceramic, polymer), metals and alloys are most commonly used for oral implants. The basic characteristic of metallic implants is their mechanical strength . As regards their behavior in a biological environment , there are metal materials with toxic tissue response (eg. Iron, nickel, cobalt, chromium, cadmium), materials that generate fibrous tissue (eg. Zinc, silver, aluminum, stainless steel, chromium – cobalt alloys) and vital reaction-inducing materials (ex. titanium, aluminum ceramic, zirconium ceramic).

Titanium is the material that allows the best osseointegration but titanium purity is different and depends on the manufacturer. Osseointegration of titanium implants means forming a rigid anchor-type connection which is accounted for by specific chemical bonds, given by the presence of oxide on titanium surface [10,11]. Modern methods of investigation, such as X-ray analysis, have shown that osseointegration is a process that takes place at the molecular level and the structure of the bone in contact with the titanium implant is that of totally mineralized lamellar, with the characteristic gaps and vital osteoblasts penetrating the porous surface of the implant. On the other hand, histological studies have revealed the trabecular bone growing directly on the surface of the titanium implant, in the case of rough porous surfaces, which favors the adhesion of fibrin, which will subsequently allow for bone apposition [12 - 16]. Therefore, to obtain a solid implant anchorage into the bone requires the presence of a biocompatible material and also an optimally designed surface of the implant (the exposed area in the mouth and the contact area with the subepithelial connective tissue are typically a processed surface) able to stimulate its bioactivity. In the bone - implant interaction bioactivity involves stimulating the osteoinductive processes (bone forming biostimulation of the cellular activity) and the osteoconductive processes (bone is so directed as to comply with the material surface). With implants of titanium and titanium alloys, the controlled modification of the surface resulted in improved bioactivity, as evidenced by the intensification of the proteins adsorption, cell interaction and cell growth at the body -biomaterials interface (Ratner and Porter,1996). Changes in the microtopography of the titanium surfaces used in dental implants through various processing techniques such as sandblasting, acid attack, or their combinations have been focused for the past 15 years on determining whether bone apposition could be strengthened through a microrough surface. The results showed that of these, sandblasted and acid-etched surface featured improved bone apposition (Buser colab,1991;Cochran and colab, 1998)

Recent research [17 - 21] has comparatively evaluated in vivo the osseointegration of titanium implants with surfaces treated by mechanical methods and chemical hydroxyapatite coating. There have been studies on the histological and osteoregenerativ process, establishing the dynamic osseointegration processes for different periods of time. The research results show that obtaining surface microretentions contribute to implant osseointegration. Due to the surface treatment with hydroxyapatite (HA) 'it was noticed a better bone apposition around the implant, especially in the early period of healing. Osteogenesis occurs on the periphery of bone defects toward the implant, in the bone interface area unformed bone tuissue being present, limited by mature bone blades". It should be pointed out that along with the topography of the area, a key role in improving the bioactivity is played by the surface chemistry (Kilpadi and Lemons, 1994) and its hydrophilic / hydrophobic balance. The hydrophilic nature of the surface characterized by high wettability in the presence of various liquids causes high speed cellular attachment. Moreover, it is known that the wettability of the material is a predictable indicator of its citocompatibility which affects both cell attachment and the degree of cell spreading onto the material. The purpose of research is the controlled changing , by acid corrosion and anodic oxidation, of the surface of Ti10Zr bio alloy, a new biomaterial recommended in oral implantology. It is a study whose results complement those obtained in the study of corrosion resistance [22] and those on assessing biocompatibility in vitro [23] of the biomaterial Ti10Zr, a high biocompatibility bio alloy obtained by strictly controlling the chemical composition (removing from composition harmful elements such as Al, V, Ni, Fe, etc., elements present in titanium alloys commonly used in dental implants).

2. Materials, methods and experimental conditions

The materials used in the experiments are samples cut from the Ti10Zr cast blanks (rings ø18mm dia). Chemical characterization is based on qualitative and quantitative chemical analysis of these samples (Table 1 and Figure 1). Microstructure analysis of the cats blank samples reveals the specific nature of casting structures characterized by large grains, uneven and chemically homogenous as illustrated by their dendritic appearance (Figure 2). Eliminating these deficiencies, which may adversely affect the technological properties and use of the product (eg. processing to obtain implant) was achieved by applying the anneal homogenisation with favourable effect on structural homogeneity and uniformity of crystal grains (aspect of microstructure is revealed in Figure 2b).

Samples as cast rings of ø18mm cut from th blank- cylinder of dimensions: l = 70mm,

Ø =18mm, were subjected to surface treatments by acid attack (etching) and anodic oxidation. Table 2 shows the experimental surface processing conditions for the material samples studied. The operation of anodic oxidation was performed in a laboratory plant in IMNR Bucharest consisting essentially of an electrolytic cell supplied from a voltage source of 150V / 10A.

Electrolyte for anodizing:

- H_2SO_4 ..... .200g/l
- NaCl..... 30g/l
- ethylene glycol..... ……...200ml

Operating parameters:

- electrolyte temperature... ……35°C
- voltage across cell..... 30V
- process duration…….. 60min

The operating continuous constant voltage was 30V.

Table 1

Chemical Composition and Mechanical Properties of the Ti10Zr [24, 25]
Compoziția chimică şi proprietățile mecanice ale biomaterialului Ti10Zr

Chemical composition / *Compoziția chimică*, %						
Elements *Elemente*	Ti	Zr	Fe	Si	Cu	Ni
[%]	88.8	9.906	0.608	0.392	0.032	0.010

Mechanical Properties / *Proprietăți mecanice*		
Densitate *Density* g/cm^3	**Hardness HV100 (cast blank) *Duritatea Vickers HV100 (semifabricat turnat) /***	**Elasticity Modulus / *Modulul de elasticitate/*, E, MPa**
4.7	212	113.000 (calculated value /*valoare calculată*)

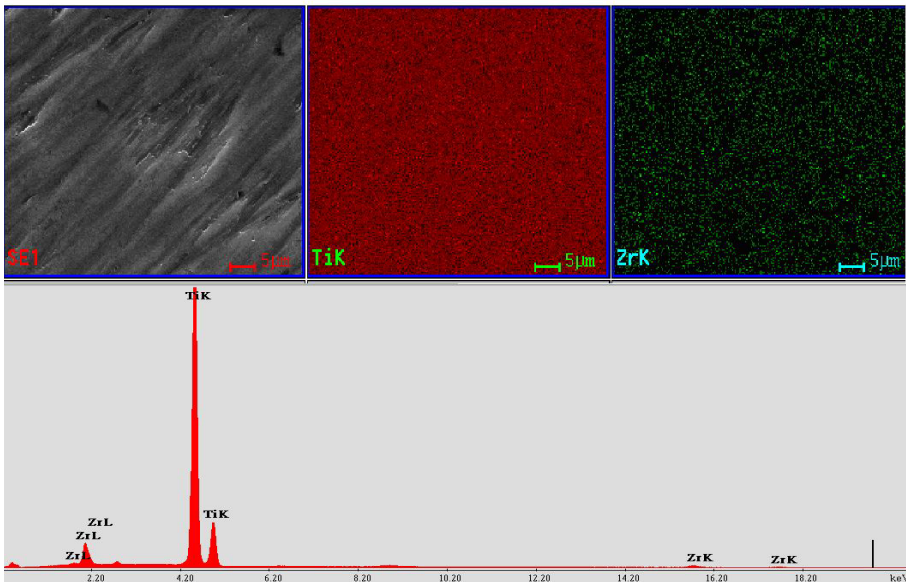

Fig.1 - Image (SEM) and EDX Spectra of samples taken from Ti10Zr alloy castings [26] / *Imaginea de microscopie electronică de baleiaj (SEM) şi spectrul EDX al eşantioanelor din Ti10Zr prelevate din semifabricat turnat [26].*

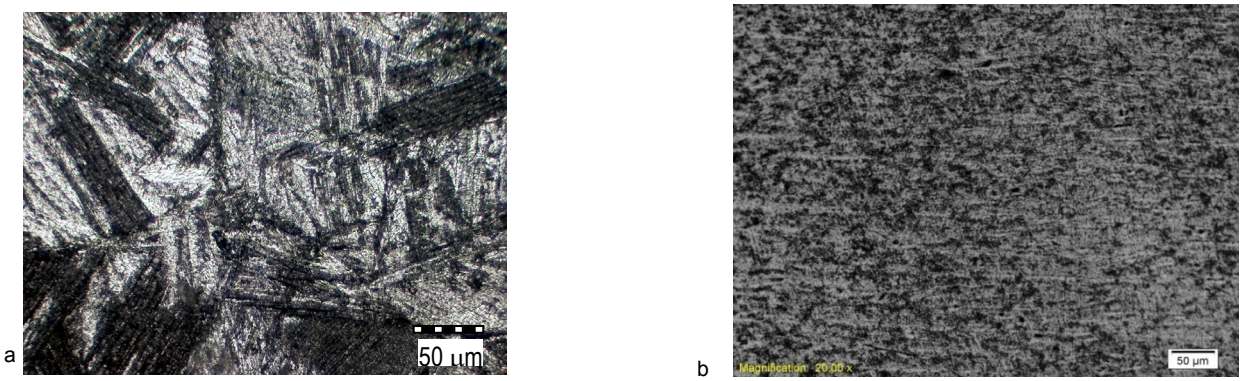

Fig.2 - Microstructural Aspects of the Samples - rings with a diameter ø18mm (optical microscopy) (a) – cast state [24]; (b) - after heat homogenization /*Aspecte microstructurale ale probelor - rondele cu diametrul ø18mm (microscopie optică) (a)-starea turnat; (b) - după recoacerea de omogenizare.*

Table 2

Experimental Conditions to Experimentally Process of the Sample Surface
Condiții experimentale la procesarea experimentală a suprafeței probelor

No.	Sample/Material Symbol *Simbol proba/material*	Pcs. *Buc.*	Surface Treatment Applied to Samples *Tratamente de suprafață aplicate probelor*
1	**G2**/ Ti-10%Zr alloy - rings ø18mm ***G2**/aliaj Ti-10%Zr rondele ø18mm*	1	Metallographic paper polish400+ degreased +rinse +dried +**acid attack*** +rinse + dried *şlefuit hârtie metalografică400+ degresat$_{us}$+spălat$_{us\ apa}$+uscat+**atac acid*** *+spălat$_{us\ apa}$+spălat$_{Aet.}$+uscat*
2	**G3**/ Ti-10%Zr alloy - rings ø18mm ***G3**/ aliaj Ti-10%Zr rondele ø18mm*	1	Metallographic paper polish400+ degreased + dried +acid attack* +rinse + dried +**anodic oxidation** *şlefuit hârtie metalografică400+ degresat$_{us}$ +spălat$_{us\ apa}$+uscat+atac acid** *+spălat$_{us\ apa}$+spălat$_{Aet.}$+uscat+**anodizat***

Amestec HCl 37%+ H_2SO_4 98%; T > 80°C (corroded)

After the operations of acid corrosion and anodic oxidation, samples were tested for evaluating the cell behavior along with samples of the same material (Ti10Zr) under rough molded and cast + surface machining condition and samples from Cp-Ti and Ti grad4 (Faculty of Biology, University of Bucharest). "In vitro" biocompatibility testing conditions for the Ti10Zr alloy focused on cytotoxicity and cell behavior are detailed in a "In Vitro Testing of Materials Biocompatibility With Controled Chemical Composition" paper [see 23].

Investigations on the nature of oxides and morphology obtained on the experimentally processed surfaces was conducted by spectral diffraction analysis (diffractometer Quanta 200 from "Dunărea de Jos" University of Galati) and scanning electron microscopy SEM (at METAV and IMNR in Bucharest).

3. Results and experiments

Figure 3 illustrates diffraction patterns obtained by X-rays spectral diffraction analysis of samples processed by acid corrosion (denoted G2) and samples with acid attack followed by anodic oxidation (denoted G3).

Scanning electron microscopic analysis of experimentally processed samples show that their surface is covered with a uniform, continuous and adherent film of titanium oxide (Figure 4, Figure 5 and Figure 6).

We can notice a very special configuration (Figure 5, Figure 7) of the surfaces processed by anodic oxidation and corrosive attack, which will be subject to further studies to determine the parameters of the biosurfaces (bioactivity, biostability, wettability, etc.) involved in stimulating biological processes that occur at the implant-tissue interface right after post-implantation.

Assessment tests of osteoblast adhesion to sample surface show good adhesion of osteoblasts on the surface of Ti10Zr cast and polished samples, cells featuring a well organized actyn cytoskeleton with cytoplasmic extensions interconnecting with neighboring cells (Figure 8).

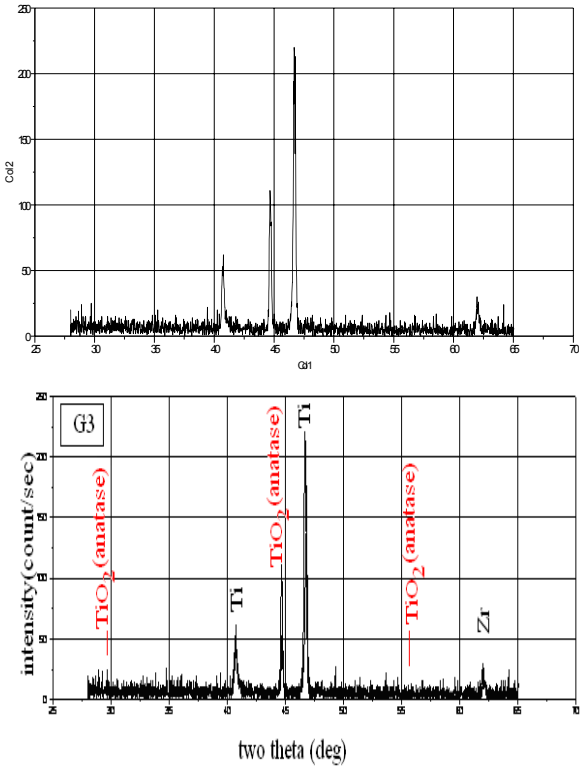

Fig.3 - Diffraction analysis of samples etched (G2) and the samples subjected to anodic oxidation (G3) / *Analiza de difracție a probelor corodate (G2) şi a probelor supuse oxidării anodice (G3).*

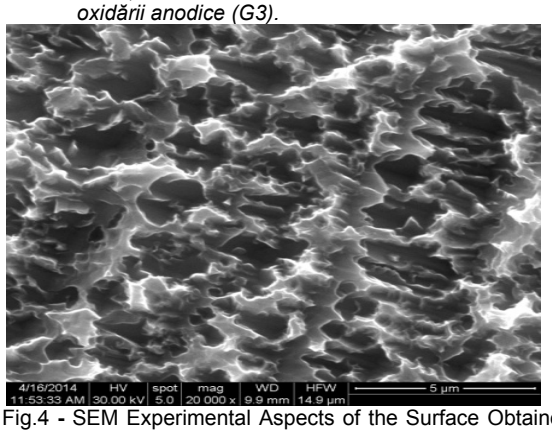

Fig.4 - SEM Experimental Aspects of the Surface Obtained by Acid Attack (etching) (X 20,000) / *Aspecte SEM ale suprafeței obținută experimental prin corodare acidă (X 20.000).*

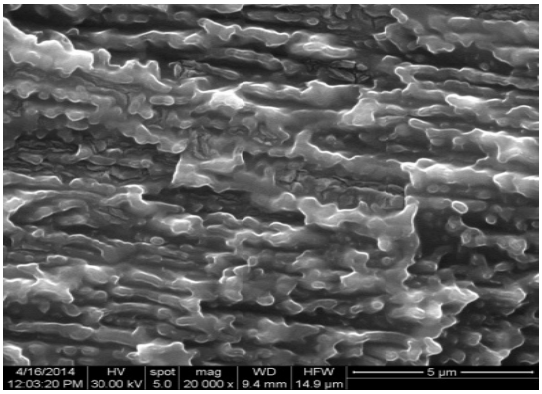

Fig.5- SEM Experimental Aspects of the Surface Obtained by Anodic Oxidation (X 20,000) / *Aspecte SEM ale suprafeței obținută experimental prin oxidare anodică (X 20.000).*

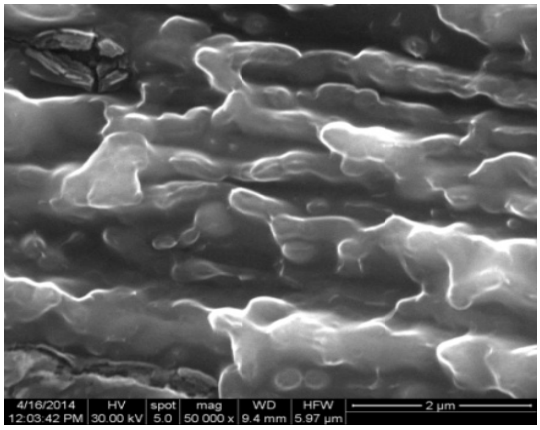

Fig.6 - SEM Aspects of the experimentally surface obtained by corroded (X 50,000) / *Aspecte SEM ale suprafetei obținută experimental: corodat (X 50.000)*

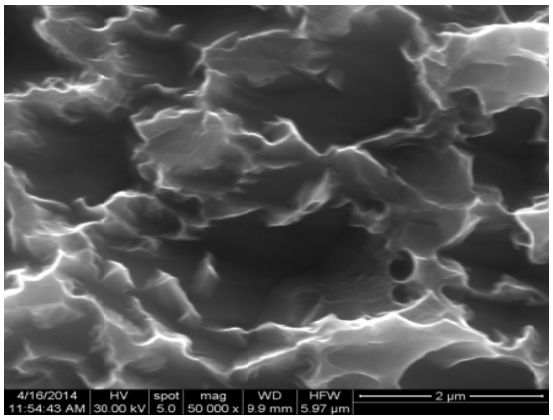

Fig.7 - SEM Aspects of the experimentally surface obtained by anodic oxidation (X 50,000) / *Aspecte SEM ale suprafetei obținută experimental prin oxidare anodică (X 50.000).*

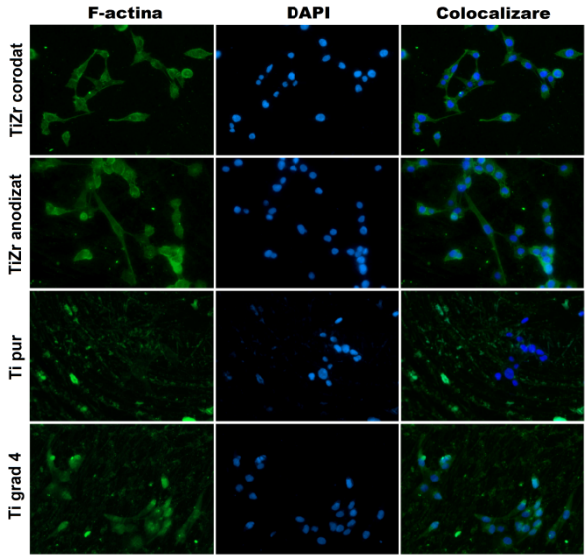

Fig. 8 - Highlighting the actin cytoskeleton by marking the F-actin fluorescence Phalloidin-FITC (counterstain nuclei with DAPI) in osteoblasts increased for 48 hours on the surface of materials Ti10Zr or pure Ti [23] / *Evidențierea citoscheletului de actină prin marcarea fluorescentă a F-actinei cu faloidina-FITC (contracolorare nuclei cu DAPI) în osteoblastele crescute timp de 48 ore pe suprafața materialelor de TiZr sau Ti pur [23]*

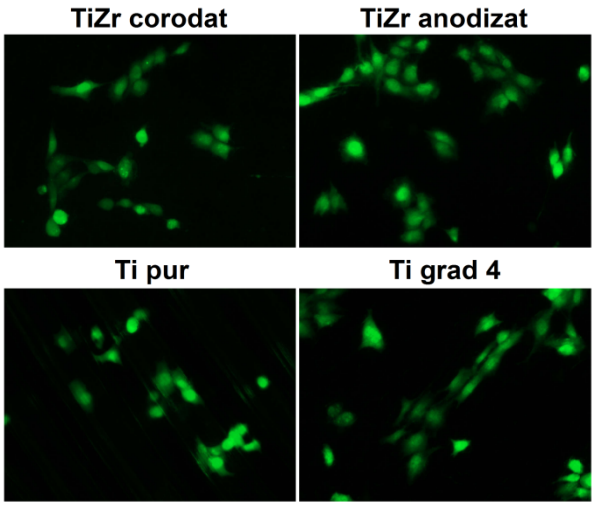

Fig. 9 - GSH distribution determined by fluorescent marking CMFDA at increased osteoblast surface for 48 hours Ti10Zr materials or pure Ti [23] / *Distribuția GSH determinată prin marcarea fluorescentă cu CMFDA la nivelul osteoblastelor crescute timp de 48 ore pe suprafața materialelor de TiZr sau Ti pur [23].*

Noteworthy is adhered cells orientation towards the direction of polishing previously carried out on Ti10Zr material surface by machining.This is also valid with the other polished materials, namely pure titanium and Ti Grade 4. It was not highlighted though for corroded or anodized TiZr, suggesting that additional machining processes have changed the properties of these surfaces, and thus influenced in a decisive manner cell orientation and adherence. These differences between materials indicate the result of adapting osteoblasts to surfaces of different properties.

Fluorescent labeling of GSH from the cells adhering to the surfaces of etched, anodized TiZr or pure titanium (Figure 9) confirmed cell adhesion and growth. The surface material

processed as described above, but also the intrinsic material properties stimulate cell proliferation after 48 hours of cultivation and provides the conditions necessary for proper cell growth.

4. Conclusions

Changing the microtopography of the xperimentally processed surfaces revealed a special configuration of the oxidized surface, especially of those obtained by anodizing. Interesting are also the results of the rough surfaces obtained by machining and evaluated by their cell response.

These results complement those obtained in the biocompatibiity study by testing the corrosion resistance and testing the cytotoxicity and "in vitro" cell behavior of the new biomaterial Ti10Zr for oral implantology. Spectral analysis with X-rays diffraction indicated the presence of TiO_2 oxides (anatase) and scanning electron microscopy analysis revealed a very special configuration of the surface oxidized by acid corrosion and anodizing.

Investigations continue with further studies on the morphology and characteristic features of the oxide layer,to establish the optimal conditions for obtaining the biosurface of the Ti10Zr bioalloy implant considered a material of high biocompatibility by eliminating from the composition the harmful elements (i.g. Al, V, Fe, Ni,...).to be found in conventional alloys and currently used in oral implantology.

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