NOI ABORDĂRI PENTRU OBȚINEREA ZIRCONIULUI OXIDAT PARȚIAL PRIN DIZOLVARE ANODICĂ
NEW APPROACH FOR PARTIALLY OXIDIZED ZIRCONIUM OBTAINING VIA ELECTROCHEMICAL ANODIC DISSOLUTION

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Present paper proposes a new obtaining method for partially oxidized zirconium used to hard tissue implants. This proposed method allows partial oxidation by anodization at different voltages. To characterize the obtained surfaces was used electrochemical impedance spectroscopy (EIS) in phosphate buffer (PBS). By SEM analysis were highlighted the differences between the original alloy microstructure and oxidized surfaces. It was pointed out the presence of a continuous and consistent oxide layer formed by anodising at a voltage of 10 V.

In terms of hardness, the oxidized surfaces presents a decrease of average value for hardness. By FT-IR spectrometry coupled with optical microscopy (OM) analysis has been proved the presence and homogenous distribution of the specific absorption band of monoclinic zirconium oxide, which confirm the presence of a large amount of oxide on the alloy surface oxidized at a voltage anodising 10 V.

The biocompatibility of obtained surfaces was done by specific tests: SBF immersion and cell viability. Demonstration of cells viability and morphology of MG-63 cell line was carried out by lactate dehydrogenase method (LDH).

Lucrarea propune o nouă metodă de obținere a zirconiului oxidat parțial folosit în ingineria implanturilor pentru țesut dur. Astfel se propune metoda de oxidare parțială prin anodizare la diferite tensiuni. Pentru caracterizarea incipientă a straturilor s-a folosit spectroscopia de impedanță electrochimică (EIS) în soluție tampon de fosfat. Prin analiza SEM au fost evidențiate diferențele microstructurale dintre aliajul inițial şi suprafețele oxidate. S-a putut observa un strat continuu şi consistent de oxid format prin anodizare la o tensiune de 10V.

Din punctul de vedere al durității, suprafețele oxidate prezintă o scădere a valorii medii a durității. Prin spectrometrie FT-IR cuplată cu microscopie optică s-a demonstrat prezența şi distribuția omogenă a benzii de absorbție specifică oxidului de zirconiu monoclinic, care susține prezența într-o cantitate mare a oxidului pe suprafața aliajului oxidat prin anodizare la o tensiune de 10V.

Proprietățile de biocompatibilitate a suprafețelor obținute s-au evidențiat prin teste specifice: imersie în SBF şi prin demonstrarea viabilității şi a morfologiei celulare a liniei celulare MG-63, prin metoda de lactat dehidrogenazei (LDH).

Keywords: *partially oxidized zirconium, anodization, biocompatibility*

1. Introduction

Taking into account the importance of hard tissue implants, the materials choosing must be severe. Biomaterials such as titanium alloys, cobalt-chrome and high density polyethylene, revolutionized the implants industry. However, there are still concerns about the adverse biological response to microscopic wear debris accumulated in the prosthetic joint space, which could lead to various complications that are undesirable in orthopedic surgery [1].

Now, due to the significant progress that has been made in medical technology, it was developed by Smith & Nephew Company a new material called oxinium (partially oxidized zirconium). Researchers [2] have demonstrated the superior properties of this material. Thanks to its hardness, surface smoothness and scratch resistance, partially oxidized zirconium is durable, showing performance characteristics superior to alternative materials [2].

Partially oxidized zirconium was developed for orthopedic applications, providing better properties than CoCr alloy, especially in terms of wear resistance, behavior during friction and biocompatibility. Prosthetic components are made of an wrought zirconium alloy (Zr - 2.5%Nb) oxidized by thermal diffusion in heated air in order to create on the surface a zirconia layer with a thickness about 5 μm [3].

The oxide layer is not externally applied; it's rather a conversion of the initial metal surface into a monoclinic zirconia layer. This oxide layer is then polished to produce a joint surface at least as smooth as a component made from CoCr.

The oxide has a perpendicular microstructure to the outer surface. It is left in a state of compressive stress, without pores or voids in the internal structure and interface. All these microstructure features inhibit crack propagation and destruction of the oxide layer when shear forces are involved, thereby enhancing the

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excellent integrity of the material [4].

Mechanical tests have shown that using partially oxidized zirconium alloy to obtain femoral components for knee prosthesis leads to considerable fatigue resistance, with values above 450 MPa for ten million cycles [5]. Unlike alumina and zirconia limitations, partially oxidized zirconium shows no brittle behavior during impact tests. Continuous loading up to 20 kN of a single condyle can cause the bending at an 45° angle without breaking and with the oxide layer attached and functional [4]. Furthermore, the monoclinic crystal structure of the oxide is not affected by the constantly exposure to autoclave sterilization. Due to its resistance, hardness and stability, partially oxidized zirconium alloy can be used to obtain prosthetic components with the same design of CoCr or other metals components [5].

2. Experimental

This paper proposes a new procedure for obtaining partially oxidized zirconium for later use in hard tissue implants engineering. Experimental, the partial oxidation of the zirconium alloy has been made using a new method, through the metal anodization at different voltages. For comparison, a sample was obtained by heating in air flow for two hours at 500°C. Pilling-Bedworth rule (P.B.) can be used to predict the nature of the oxide layer formed on a metal surface by calculating a coefficient using the next formula [6]:

$$P.B.C. = \frac{\text{Oxide molar value}}{\text{Metal molar value}} = \frac{M_{Ox} \cdot \rho_{Me}}{n \cdot M_{Me} \cdot \rho_{Ox}}$$

where n = the number of metal atoms / 1 mole of oxide.

According to Pilling-Bedworth coefficient, if its value is smaller than 1, the oxide layer is thin and can be easily detached from the metal surface; if it's bigger than 2, the layer will come off and will not protect the metal substrate. If the value is between 1 and 2, the oxide layer will be consistent, non-porous and thus protect the metal surface. Zirconium P.B. coefficient is 1.56, which allows the use of electrochemical anodization for partial oxidation of zirconium alloy [6].

Passivity is a state of increased resistance to corrosion under certain conditions in which the metal is not thermodynamically active due to the formation of a stable film that inhibits the anodic dissolution of the metal. The passivity may occur when the metals are submerged in solutions made from oxidizing agents (auto-passivation) or when the metals are anodic polarized in suitable electrolytes. This treatment leads to the formation and growth of oxide films on metal surfaces [7].

Thus, samples of niobium-zirconium alloy with surface (S) of 2.3 cm^2 were processed prior to experimental measurements by cleaning with emery paper, washed and rinsed with double distilled water, degreased in ultrasonic bath in acetone for 300 seconds.

Electrochemical system consisted of zirconium alloy sample as working electrode (anode), a platinum electrode (S = 1.13 cm^2) as auxiliary electrode (counter-electrode) and as a reference it was used an Ag, AgCl-KCl sat. electrode.

Measurements were carried out using a potentiostat/galvanostat Voltalab 40 connected to a computer. The measurements were performed at room temperature, 25°C.

3. Results and discussion

3.1. Electrochemical characterization of the oxide film

Electrochemical impedance spectroscopy (EIS) in phosphate buffer (PBS) was realized to verify the characteristics of the film obtained by anodization. Phosphate buffer is a solution commonly used in biological studies because it matches with those from the human body. The used solution had the composition: NaCl 137 mM/L, KCl 2,7 mM/L, Na_2HPO_4 10 mM/L şi KH_2PO_4 1,8 mM/L.

Impedance tests results for the samples are presented as Nyquist and Bode diagrams in Figure 1. The behavior of the surface changes with the formation of oxide film and film properties differs depending on the electrical potential value used for anodization.

General behavior of zirconium alloy is represented by the increase of surface impedance with anodization and with potential growth. At a potential value of 10 V the sample presents a capacitive behavior. For zirconium alloy and zirconium alloy oxidized at 5 V samples, systems behavior shows a combination of kinetic and diffusion processes. Nyquist diagrams shows the presence of a single time constant for sample oxidized at 10 V (noted Zr 10V), while in the case of zirconium alloy and zirconium alloy oxidized at 5 V (noted Zr 5V) it can be observed the presence of two time constants, indicating a change in the mechanism with the increase of anodizing potential.

The increase of the phase angle for the zirconium oxidized at 10 V sample is the proof of the capacitive behavior of the oxide film from the alloy surface.

EIS showed low charge transfer characteristics for the sample anodized at 10 V compared to the zirconium sample and the sample anodized at 5 V.

The intersection of the curve with ZRe axis at high frequencies corresponds to ohmic resistance, which is the total resistance due to the electrolyte, separators and electrical contacts. The semicircle in the middle frequency domain (which occurs in the Zr sample) indicates the charge transfer resistance and the linear zone in the low frequency domain is the Warburg impedance, which represents the influence of the diffusion

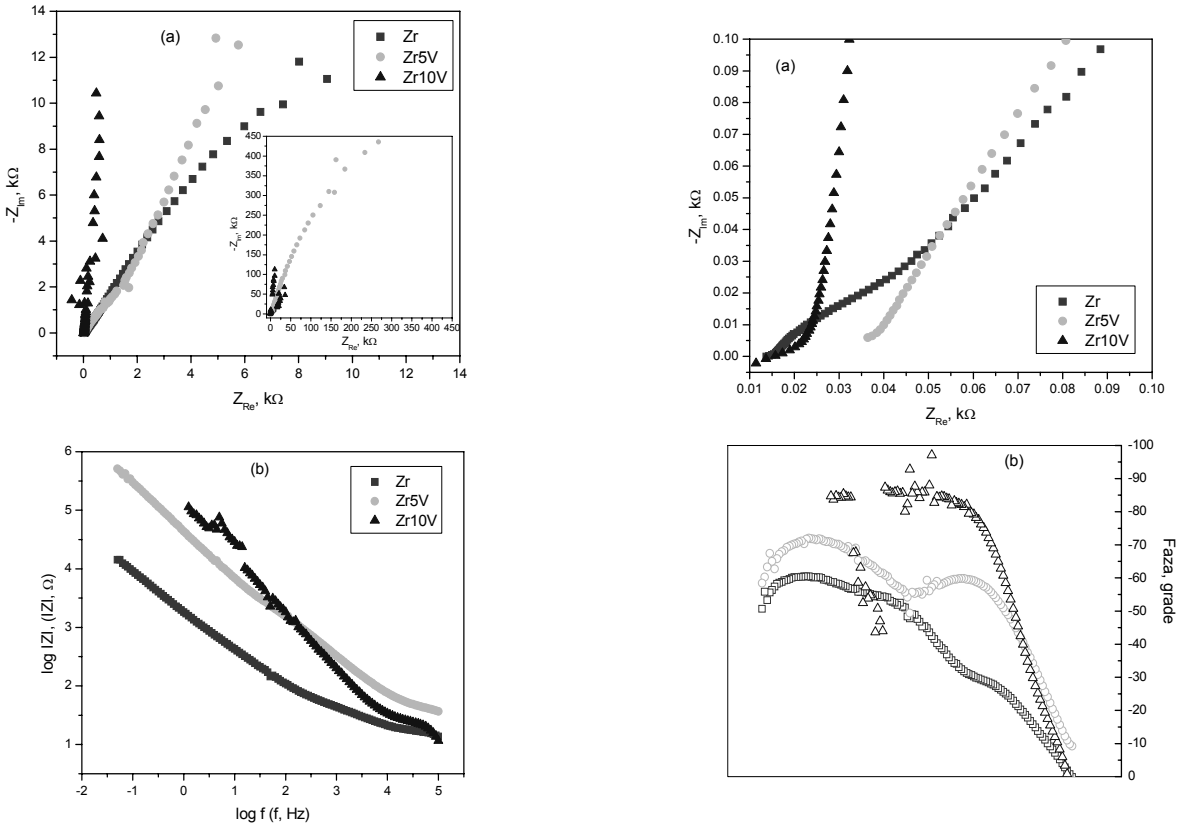

Fig. 1- Nyquist diagrams (a) and Bode diagrams (b) for Zr, Zr 5 V and Zr 10 V samples / *Diagramele Nyquist (a) şi Bode (b) pentru probele de Zr, Zr 5V şi Zr 10V.*

Table 1

Electrochemical parameters of the Zr, Zr 5 V and Zr 10 V samples in PBS
Parametrii electrochimici pentru probele Zr, Zr 5V şi Zr 10 V în PBS

Sample *Proba*	Nyquist Representations / *Reprezetarea Nyquist*		Bode Representations / *Reprezentarea Bode*
	R_{ct}, kOhm	C_{dl}, µF	log |Z|, (Z, ohm) (at 1 Hz frequency)
Zr	224	3,18	3.26
Zr 5V	1499	2.12	4.64
Zr 10V	-	-	5.22

process. The decrease of charge transfer resistance is represented by an improvement in charge transfer kinetics. The lower resistance of the sample obtained at 5 V can be associated with the formation of a porous film.

Impedance data were analyzed with VoltaMaster software and the computed parameters are shown in Table 1.

As shown in Table 1, for the sample oxidized at 10 V, the values of charge transfer resistance and double layer capacity were not found, due to the capacitive behavior of the curve showing a sharp increase in the value of imaginary resistance.

3.2. The characterization of partially oxidized surface

3.2.1. Microstructural studies

The microstructure was analyzed by scanning electron microscopy, using a QuantaInspect F scanning electron microscope (1.2 nm resolution).

From the Figures 2-5 it can be observed the differences in microstructure between the initial alloy and the three cases considered to oxidation. It can be seen that a continuous and consistent oxide layer is obtained when the sample is anodized at a voltage of 10 V. Qualitatively, the oxide layer obtained by conventional treatment (oxidation in air flow) seems to be the weakest.

Also, the EDX spectra sustain the oxide formation, on treated samples being visible the oxygen peak.

3.2.2. Vickers hardness

The hardness is a physical and mechanical property and represents a material puncture resistance to another material with a composition harder than the studied one. To determine the Vickers hardness it was used a Shimadzu HMV equipment and a force HV1 – representing 9.8 N equivalent to 1 kg and 15 seconds press time. In Table 2 are presented Vickers hardness HV1 and density values HRC.

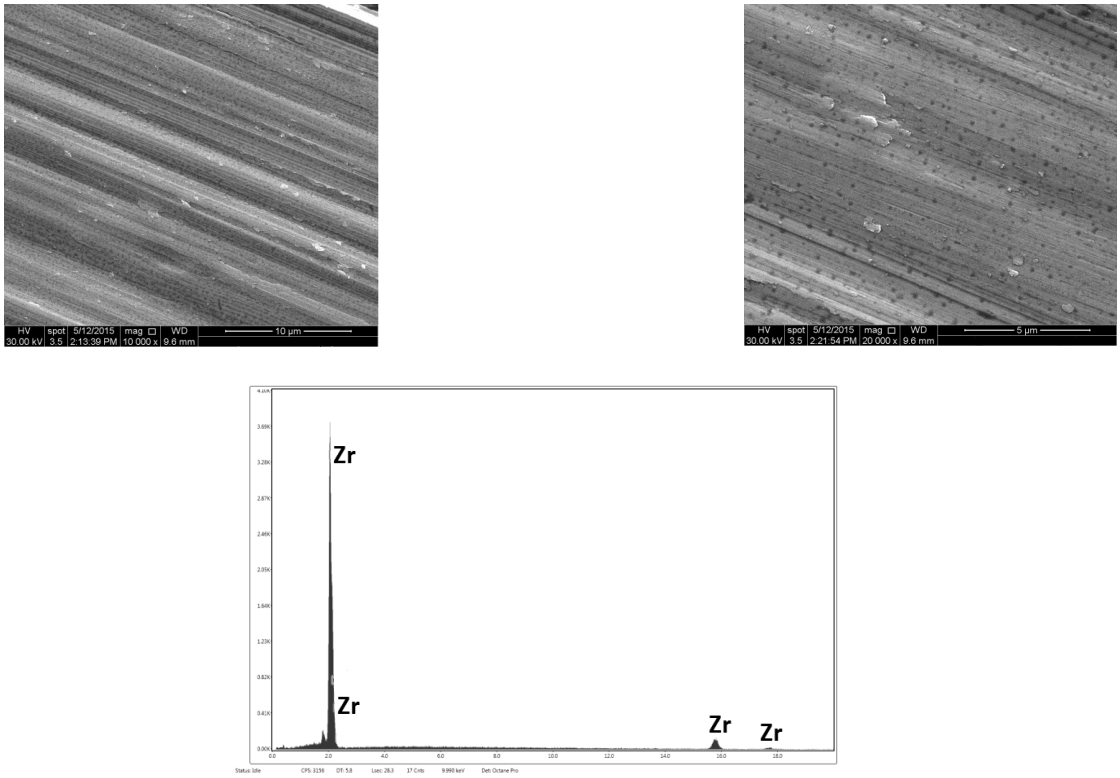

Fig. 2 - SEM micrographs (A) and EDX spectrum (B) of the zirconium alloy / *Micrografii SEM (A) şi spectrul EDX (B) ale aliajului de zirconiu.*

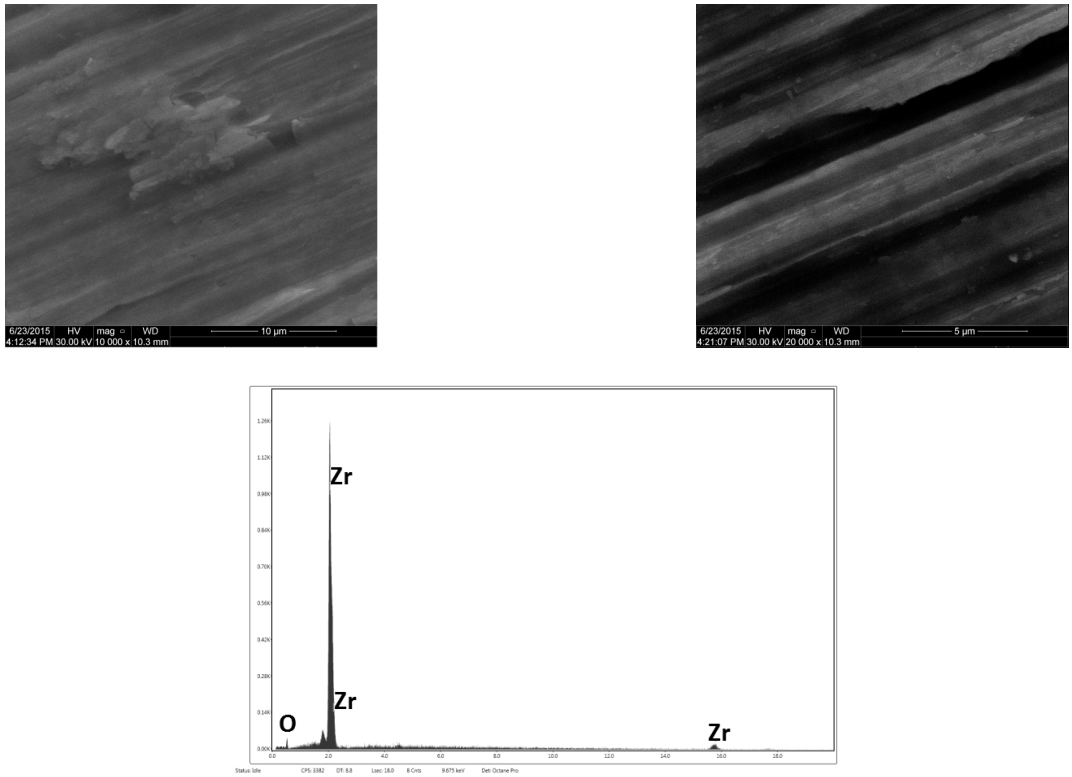

Fig. 3 - SEM micrographs (A) and EDX spectrum (B) of the partially oxidized in air flow at 500°C/2h zirconium alloy / *Micrografii SEM (A) şi spectrul EDX (B) ale aliajului de zirconiu oxidat parțial în flux de aer la 500°C/2h.*

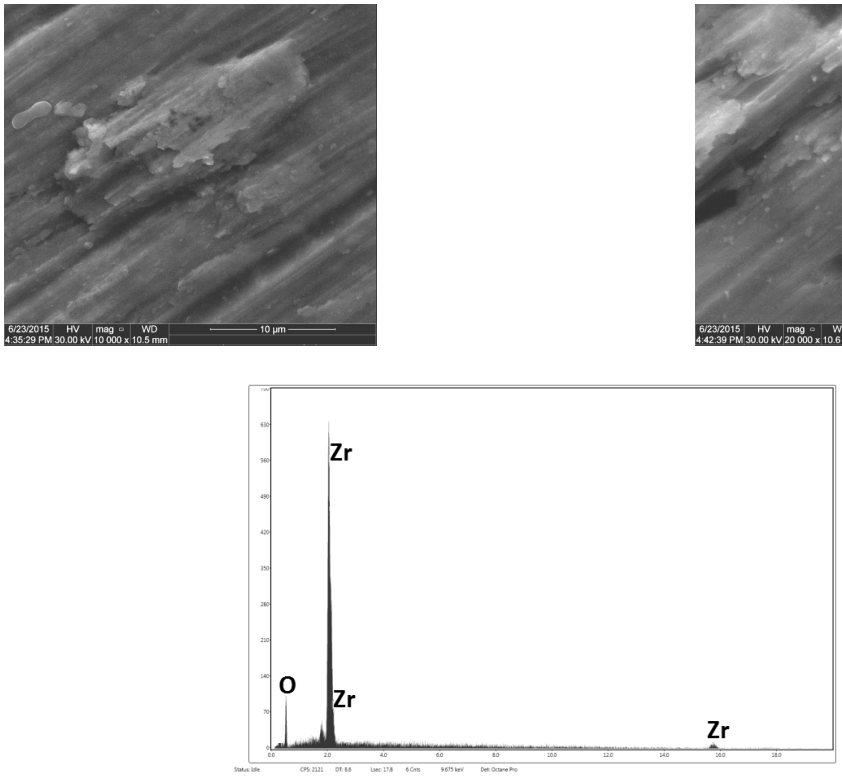

Fig. 4 - SEM micrographs (A) and EDX spectrum (B) of the zirconium alloy anodized at 5 V/ *Micrografii SEM (A) şi spectrul EDX (B) ale aliajului de zirconiu oxidat parțial prin anodizare la 5V*.

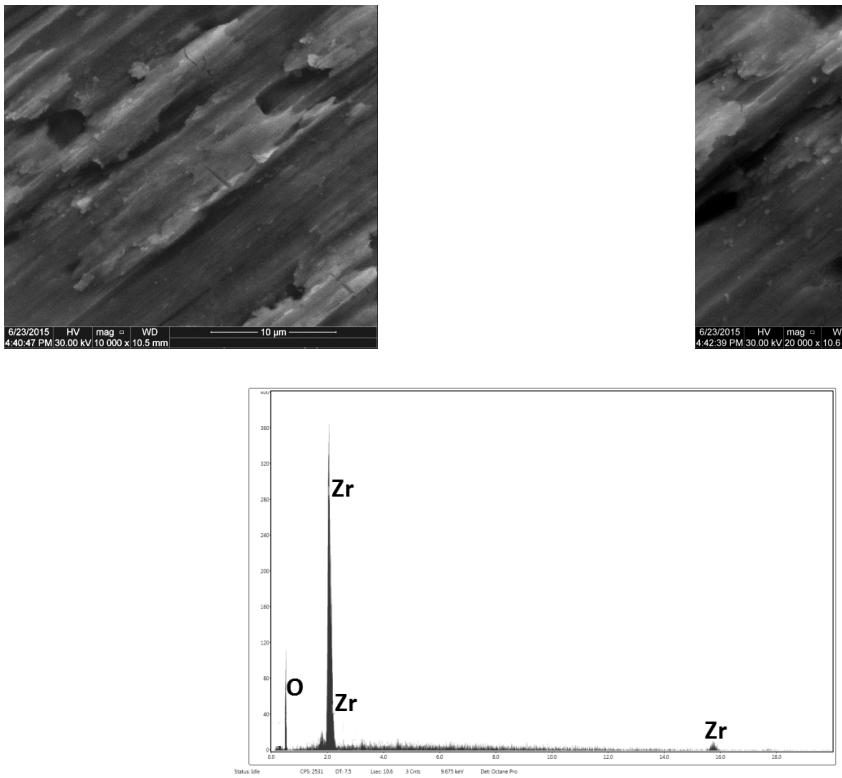

Fig. 5 - SEM micrographs (A) and EDX spectrum (B) of the zirconium alloy anodized at 10 V/ *Micrografii SEM (A) şi spectrul EDX (B) ale aliajului de zirconiu oxidat parțial prin anodizare la 10V*.

Table 2

Vickers hardness HV1 and density HRC / *Duritatea Vickers HV1 şi densitatea HRC*

	Hardness / *Duritate*			Hardness / *Duritate*	
	HV1	HRC		HV1	HRC
	255	23.4		190	8.5
	255	23.1		201	11.2
Zr	254	23	Zr 500°C	201	11.2
	253	22.8		175	4.5
	253	22.1		201	11.2
	Average / *Medie*			Average / *Medie*	
	254	22.88		193,6	9.32
	Hardness / *Duritate*			Hardness / *Duritate*	
	HV1	HRC		HV1	HRC
	241	20.6		231	18.2
	227	17.2		226	17.6
Zr 5V	210	13.2	Zr 10V	224	16.5
	215	14.4		223	15.7
	220	15.5		239	20.2
	Average / *Medie*			Average / *Medie*	
	222.6	16.18		228.6	17.64

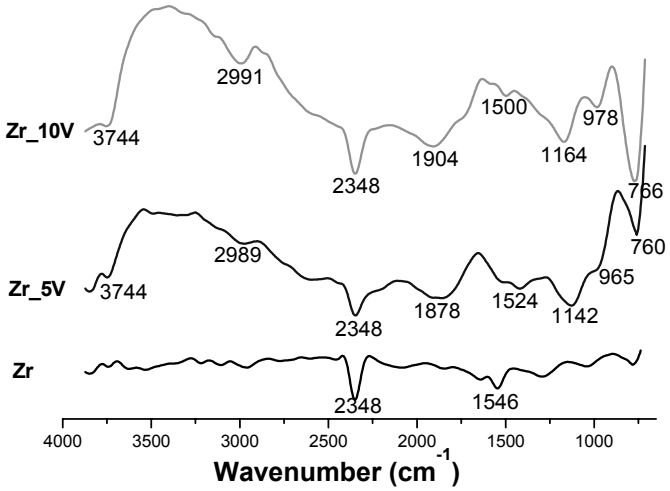

Fig. 6 - Infrared absorption spectra for Zr, Zr 5V, Zr 10 V / *Spectrul de absorbție în infraroşu pentru probele Zr, Zr 5V şi Zr 10 V.*

It can be observed that on the oxidized surfaces, whatever the used method, surface phenomena occurred, decreasing of the average value of hardness. The decrease is more pronounced for conventionally treated surface, which supports the electronic microscopy images from above.

3.2.3. FT-IR spectrometry coupled with optical microscopy (OM) analysis

For FT-IR spectrometry coupled with optical microscopy (OM) analysis, a FT-IR Thermo iN10-MX microscope was used.

For the surfaces proved to be better oxidized (those obtained by anodization) and the untreated alloy was determined the infrared absorption spectra, shown in Figure 6.

Absorption bands detected in the spectra are allocated as follows:

- at 760 and 766 cm^{-1} – the vibration characteristic monoclinic zirconium oxide (zirconium oxide remaining species show specific absorption bands in IR at smaller wavenumbers);
- large bands at 1164 and 1142 cm^{-1} occur due to the stretching vibration of the Zr-OH bond from $Zr(OH)_4$;
- weak and large absorption bands with peaks in 3000 – 3400 cm^{-1} area are due to stretching vibration of O-H group from the molecular water adsorbed or from the hydroxyl groups with a wide range of hydrogen bonds (ν_1 and ν_3 types);
- absorption band at 3744 cm^{-1} is characteristic to the vibration of Zr-OH deformed bond;
- absorption effect at 2990 cm^{-1} is characteristic to the vibration of interstitial molecular water;

- the absorption effects from 1550 cm^{-1} and 2348 cm^{-1}, belongs to the vibration characteristic to Zr-Zr bond from the initial zirconium alloy structure.

presence in a large amount on the alloy surface.

3.2.4. Biocompatibility tests

Quantification of the biocompatibility of the

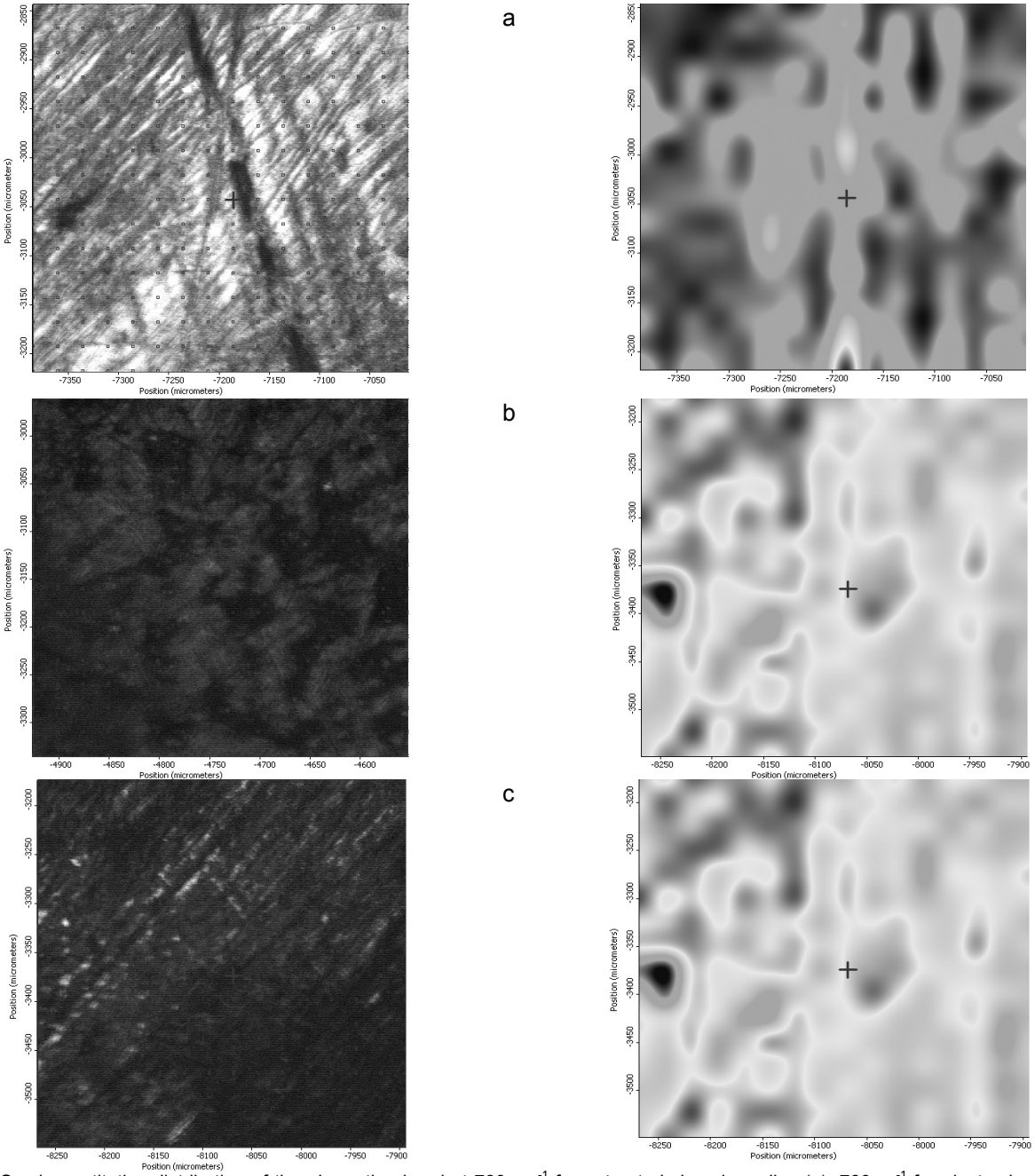

Fig. 7- Semi quantitative distribution of the absorption band at 760 cm^{-1} for untreated zirconium alloy (a), 760cm^{-1} for electrochemically treated alloy at 5V (b) and 766 cm^{-1} for electrochemically treated alloy at 10V (c) / *Distribuția semicantitativă a benzii de absorbție la 760 cm^{-1} pentru zirconiu netratat (a), 760 cm^{-1} pentru aliajul tratat electrochimic la 5V (b) şi 766 cm^{-1} pentru aliajul tratat electrochimic la 10V (c).*

Because certainly, the band at about 760cm^{-1} belongs to the zirconium oxide, using OMNIC Picta software it can be made semiquantitative distribution maps of this type of bond – Figure 7.

From the distribution of colors, it can be observed that the anodized surface at 10 V has a higher share of the absorption band specific to monoclinic zirconium oxide, which reveals its

surfaces obtained was done by specific tests: immersion and retention in SBF and cell viability tests.

3.2.5. Immersion and retention in SBF

The samples previously obtained were immersed in 10ml of SBF and up to 7 days the pH and conductivity were monitored. Results are shown in Figure 8. At small intervals, up to 12 h, it

can be seen a rapid change both of pH and ionic conductivity, followed, after 7 days, by a stabilization around a value of equilibrium.

The samples were further stored in SBF up to 14 days, after which they were immersed in distilled water for 6 h to remove any soluble salts deposited on the surface, and finally they were dried at 60°C for 24 h. After these steps, the probes were examined with a electron microscope.

From SEM images (Figure 9) for samples kept in SBF for 14 days, it is noted that the sample obtained by anodization at 10 V has a superior behavior, the amount of calcium phosphate deposited on the surface is superior than the sample anodized at 5V. Also, by EDX elemental analysis we can obtain the ratio Ca/P. For the sample anodized at 5V, the ratio is 0.65 and for the sample anodized at 10 V, the Ca/P ratio is 0.88. Both values are below 1.66 (corresponding hydroxyapatite), which supports the idea that the deposits observed are actually mixtures of calcium phosphates, which later will turn into hydroxyapatite (HAp).

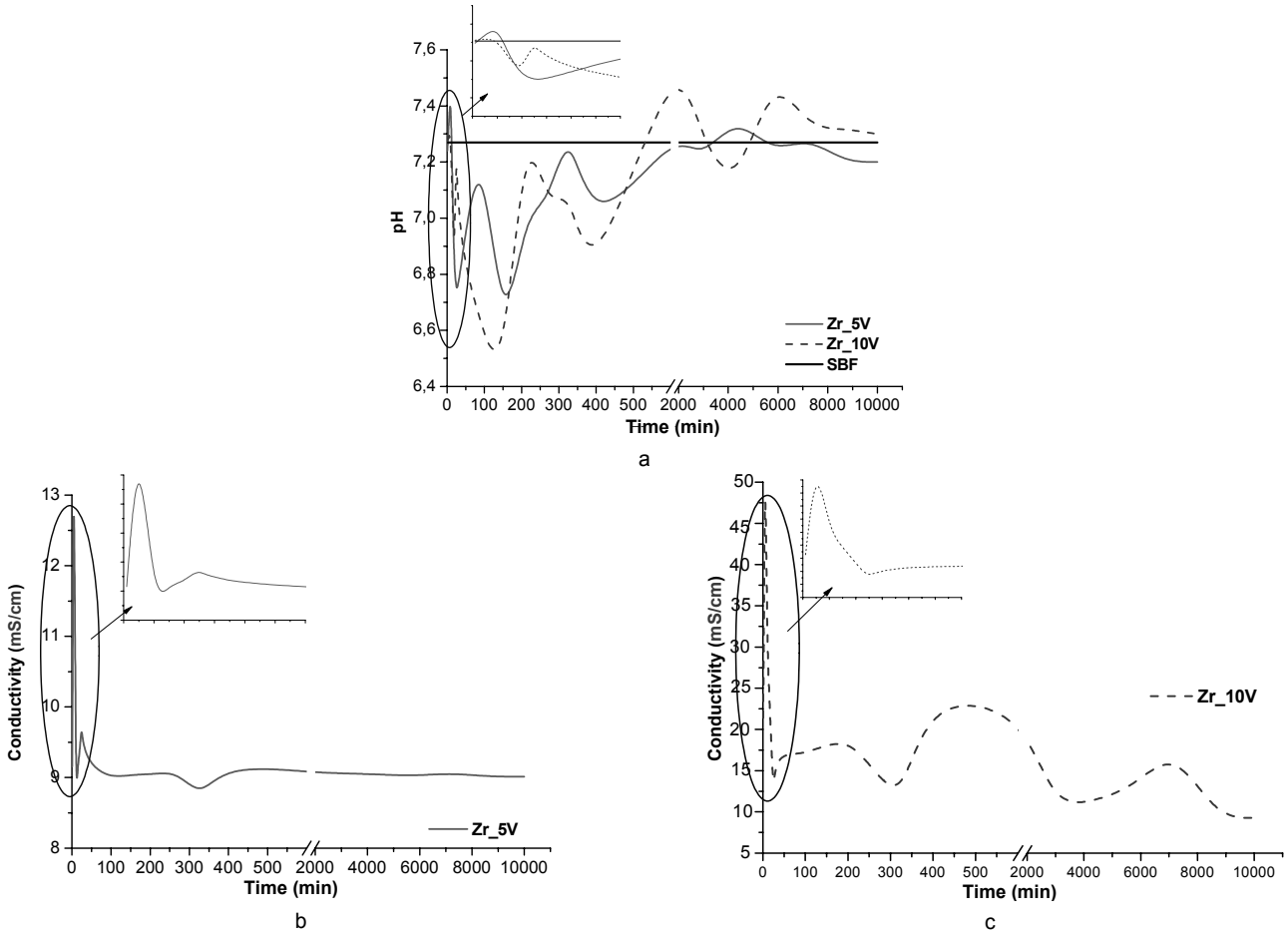

Fig. 8 - The variation of pH (a) and ionic conductivity (b,c) of the samples electrochemically treated at 5V and 10V / *Variația pH-ului (a) şi a conductivității ionice (b,c) pentru aliajul de zirconiu tratat electrochimic la 5V şi 10V*.

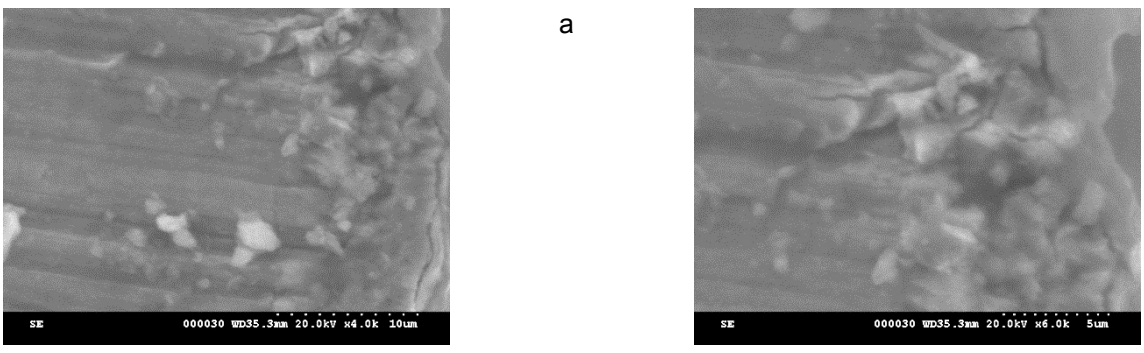

Figure 9 continues on next page

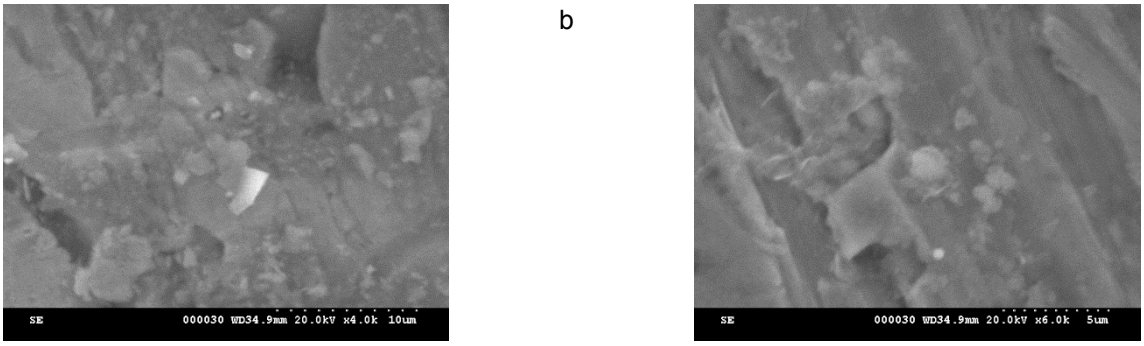

Fig. 9 - SEM imagines of the sample electrochemically treated at 5V (a) and at 10V (b), after 14 days in SBF / *Imagini SEM pentru probele obținute prin anodizare la 5V (a) şi la 10V (b) după 14 zile menținere in SBF.*

3.2.6. Cell viability tests

For determination of the viability of the cell line MG-63 was used lactate dehydrogenase method (LDH). The MG-63 cells are a line derived from osteoblasts, with similar morphology with fibroblasts and grows in monolayer. Lactate dehydrogenese is a cytosolic enzyme that is released in the culture environment as a result of cellular integrity losing, which results in apoptosis or necrosis. The LDH enzyme catalyses the conversion of lactic acid in pyruvic acid and it is a marker for cellular toxicity in biocompatibility testing. LDH activity can be used as an indicator of the integrity of the cell membrane and serves as a general method for the assessment of cytotoxicity results for chemical compounds, environmental factors or toxic materials.

The monolayer of MG-63 line were approximately 70-80% confluent at 24 h after cultivation in wells. The solutions prepared for testing cells were of 1 mg/mL concentration and incubated for 24 h.

Endothelial cell lines MG-63 were grown in 6-well plates with a seeding density of 75,000 cells/well in the presence of various biomaterials. After 24 h incubation with the materials of interest, the supernatants were taken for measurements of LDH. A 50 uL aliquot was transferred to a 96 well plate and added the reactants, according to the following protocol: 50 µl of Solution 1 is added and incubated at room temperature in the dark for 30 minutes, a volume of 50 µl of Solution 2 was added and incubated for 1 h and after is measured with Mitra spectrophotometer at a wavelength of 450nm.

When calculating the viability, both positive and negative control are taken in consideration, also it have been draw the calibration curve on the interval of interest. From the values of cell viability for each material tested emerge that LDH activity was calculated as the amount of environmental activity of cells treated divided by the activity of cells treated with lysis solution (killed 100%) (control).

The control used was MG-63 cell line incubated under the same conditions as the cells on which the materials of interest were tested. They were incubated in DMEM medium without FBS for 24 h and incubated in parallel under the same conditions as the group treated cells.

The results shows that exposure of MG-63 cell line to partially oxidized zirconium alloy for 24h leads to an low activity of LDH (33% and 29%) and the viability percentage linked to this it was 67% and 71%, respectively for samples electrochemically treated at 5V and at 10V (Figure 10).

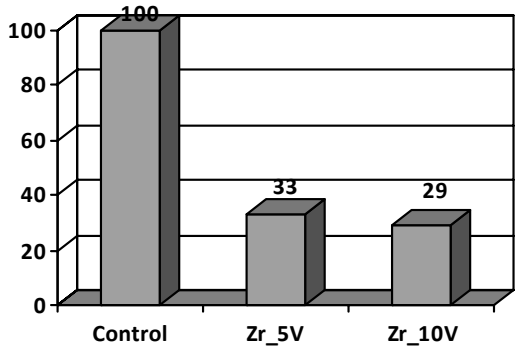

Fig. 10 - The amount of LDH released into the culture medium for samples anodized at 5V and 10V / *Cantitatea de LDH eliberată în mediul de cultură pentru probele anodizate la 5V şi 10V.*

In comparison, it is found that materials based on zirconium alloy partially oxidized, induce a significant cell viability.

4. Conclusions

In present paper it was proposed a new method for the oxidation of zirconium alloys – electrochemical anodization at different voltages. The results of the experimental test carried out on oxidized alloy by the conventional method and the method proposed in this paper, revealed the following:

- The impedance tests reveal that the alloy surface changes its behavior once with obtaining the oxide film and the properties of the films differ depending on the value of the potential used for anodizing. It was also noted that the sample under anodization at 10 V potential shows a capacitive behavior.

- Microstructural studies lead to the idea, that anodization at 10 V is superior to other methods (conventional treatment and anodizing at 5 V), because it determines grow of a consistent form and continuous layer of oxide.
- Through the Vickers method, it was noticed that certain surface phenomena occur which lead to lower hardness values, the lowest value being in the case of conventional treated piece. Thereby, we can conclude that, although the values are reduced compared to the original piece, the anodization increases the hardness more than the method used in current practice.
- FTIR analysis coupled with MO reinforces the idea that anodized surface at a voltage of 10 V has a superior behavior than the other probes, showing a higher proportion of distribution band absorption specific for zirconium oxide monoclinic.
- By quantifying the biocompatibility it was noticed that the sample obtained by anodizing at 10 V has a superior behavior.

The tests performed support the idea that the surface obtained by anodizing at 10 V is superior to that obtained conventionally by heat treatment at 500°C/2h.

Therefore, we conclude that anodization adds a plus in the field of partial oxidation of oxygen flow and in the case of properties for the obtained materials, which are clearly superior.

Acknowledgment

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Când am luat moştenirea întemeietorului României moderne, am făgăduit înaintea reprezentanţilor naţiunii că voi fi un bun român. Cred că m-am ţinut de cuvânt.
Grele au fost timpurile, mari au fost jertfele, dar strălucită e răsplata, şi astăzi pot spune cu fruntea senină: faţă de Dumnezeu şi faţă de poporul meu am conştiinţa curată.

Ferdinand, Regele României

Românii nu vor putea uita niciodată că regele Ferdinand s-a sacrificat pentru binele ţării. De neclintit în convingerile şi în hotărârile sale în timpul războiului, bun şi înţelept pe timp de pace, Ferdinand va rămâne pentru totdeauna regele care s-a identificat cu poporul său şi care a înfăptuit marile reforme care au adus statului dreptatea, puterea şi liniştea.

Ion I. C. Brătianu

(vezi Recenzie pag. 301)

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