IN MEMORIAM Prof. Dr. Ing. PETRU BALTĂ

INFLUENȚA Y_2O_3 ŞI ISTORIEI TERMICE ASUPRA CERAMICILOR ZIRCONICE DENTARE
THE INFLUENCE OF YTTRIA AND THERMAL HISTORY ON DENTAL ZIRCONIA CERAMICS

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The aim of this study is to investigate the influence of different thermal histories and yttrium oxide molar ratio on zirconia dental ceramics properties. Zirconia powders used in this study were stabilized with 2 mol%, 2.5 mol% and 3 mol% yttrium oxide. Final dense ceramics were obtained through hot isostatic pressing at temperature of 1300°C, plateau of 1 and 2 hours, under 150 MPa, in argon atmosphere. Phase composition and microstructure were determined through X-ray diffraction (XRD) and scanning electron microscopy (SEM). Density of specimens was investigated through gas displacement method on a helium pycnometer. Compressive strength and Young's modulus tests were also performed in order to evaluate the effect of HIP treatment. Finally, the ceramics were tested for biocompatibility, showing good results.

Scopul acestei lucrări este de a studia influența istoriilor termice diferite şi a raportului molar al oxidului de ytriu asupra proprietăților ceramicilor zirconice dentare. Pulberile zirconice folosite în acest studiu au fost stabilizate cu 2 mol%, 2,5 mol% şi 3 mol% oxid de ytriu. Ceramicile dense finale au fost obținute prin presare izostatică la cald, la temperatura de 1300°C, palier de 1 şi 2 ore, presiune 150 MPa, în atmosferă de argon. Compoziția fazală şi microstructura au fost determinate prin difracție de raze X şi microscopie electronică de baleiaj. Densitatea probelor a fost investigată prin metoda dizlocuirii cu gaz la un picnometru cu heliu. Rezistența la compresiune şi modulul lui Young au fost şi ele investigate pentru a determina influența tratamentului HIP. La final ceramicile au fost testate pentru biocompatibilitate, oferind rezultate bune.

Keywords: *ceramics, density, compressive strength, biocompatibility, dental*

1. Introduction

Zirconia is a valuable ceramic thanks to its transformation-toughening mechanism. The increase in volume which occurs causes compressive stresses and limits crack propagation in material [1,2]. Due to its superior properties this material has a wide range of application, starting with structural applications such as extrusion dies, cutting tools, valve guides and ending with its use like a biomaterial [2-4]. Monoclinic zirconia is stable below 1170°C, tetragonal zirconia between 1170°C-2370°C and cubic phase is stable above 2370°C [1,4,5]. In order to make it suitable for applications different oxides, such as Y_2O_3, MgO, CeO_2 are used to stabilize zirconia [6,7]. In dentistry, zirconia implants are becoming increasingly on the strength of its mechanical properties, biocompatibility and superior aesthetics [7]. Zirconia crowns or bridges have a much longer life span than metallic-based restoration (lifetime vs. more than twenty years)[3].

The objective of this study is to evaluate the influence of thermal history and yttrium oxide on phase transformation, mechanical properties and density of zirconia dental ceramics. They were used three different molar ratios of yttria (2, 2.5 and 3 mol%) and three different thermal histories.

2. Materials and Methods

2.1.Preparation of zirconia ceramics

The starting zirconia powders stabilized with 2, 2.5 and 3 mol% yttrium oxide, were obtained through sol-gel method. The synthesis of tetragonal zirconia is described in another paper [8]. The ceramics processing flowchart is presented in Figure 1.

After uniaxial pressing on a hydraulic press (Carver, model 4350.L), the specimens were hot isostatic pressed at 1300°C, for 1 and 2 hours, under 150 MPa, with a heating rate of 5°C/min and a cooling rate of 20°C/min, after the following thermal histories:

- HIP 1: specimens were directly hot isostatic pressed.

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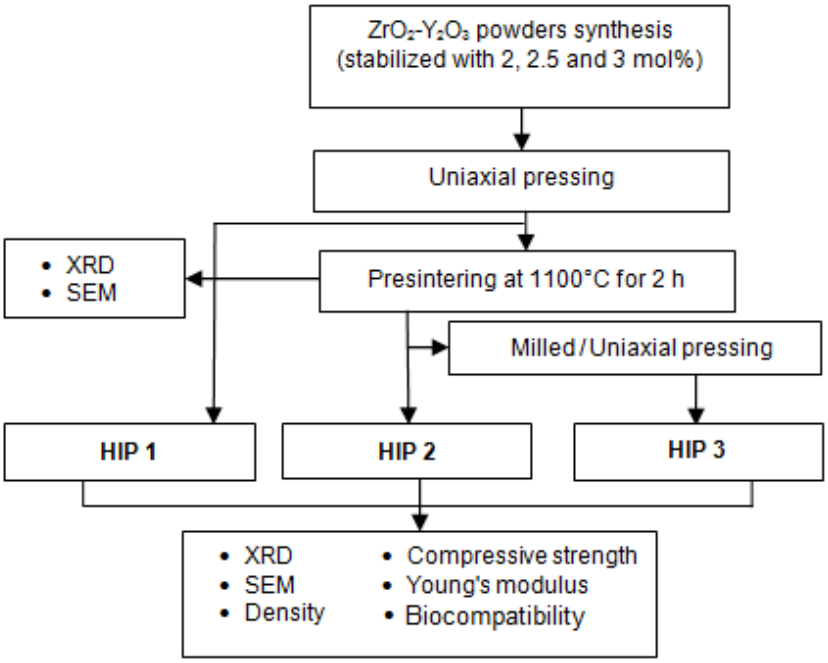

Fig. 1 - Processing flowchart / *Fluxul tehnologic.*

- HIP 2: specimens were first pre-sintered in air at 1100°C for 2h (with a heating rate of 5°C/min and cooled as rapidly as possible) and then they were hot isostatic pressed.
- HIP 3: specimens were pre-sintered in air at 1100°C for 2h (with a heating rate of 5°C/min and cooled as rapidly as possible) and then they were milled with a mortar and pestle and again uniaxial pressed, process followed by hot isostatic pressing.

The cooling rate of 20°C/min was chosen to avoid tetragonal to monoclinic phase transformation and it was the maximum cooling rate that the hot isostatic press could support. Further specimens will be referred to as 2ZY, 2.5ZY and 3ZY, where 2, 2.5 and 3 represents the molar ratio of yttria.

2.2. X-ray diffraction

X-ray diffraction analysis of powders and sintered samples was performed on a Panalytical Empyrean diffractometer (step size 0.02°, time per step 1 sec) at room temperature. In all the cases, Cu Kα radiation with λ=1.541874Å was used. The samples were scanned in the Bragg angle 2θ range of 10-80 deg.

2.3. Scanning Electron Microscopy

For the microstructure analysis of powders and sintered samples was used an INSPECT F50 scanning electron microscope, with field emission gun (FEG, resolution – 1.2 nm) and X-ray spectrometer (EDS, resolution at MnK of 133 eV), on samples covered with a thin gold layer.

2.4. Ceramic properties

The density of sintered specimens was determinated by Archimedes' principle of gas displacement using a helium pycnometer (Pycnomatic model). The compressive strength and Young's modulus were determinated using a testing machine (INSTRON model 5982, with a Blue Hill 3 soft).

2.5. Biocompatibility

2.5.1. Cells

MG63 line (ECAC90032006) used in our experiments, was cultivated in DMEM:F12 (Gibco, NY, SUA) supplemented with 10% heat-inactivated bovine serum and penicillin (100U/ml), streptomycin (100µg/ml). The cells were maintained at 37°C with 5% CO_2 and humid conditions.

2.5.2. Cytotoxicity Assay

In order to determine cytotoxicity, approximately $7x10^4$MG63 cells were seeded in 24-wellplate and after 24h were treated with 1mg/ml of zirconia based ceramics. After 24h, the cells were observed at inversed microscope, photographed in contrast phase with Zeiss Observer D1 microscope and stained with 10µg/mL Hoechst and 5µg/mL propidium iodide. The stained cells were visualized in fluorescence with Leica DFC450C microscope and photographed. On the other hand, another well treated with 1mg/mL of the zirconia based ceramics were used to perform Trypan blue stain evaluation. Briefly, a freshly prepared solution of 50 µL Trypan blue (0.05% in distilled water) was mixed to 50 µL of each harvested cellular suspension during 5 min, spread onto a

microscope slide and covered with a cover slip. Nonviable cells appeared blue-stained. At least 200 cells were counted per treatment.

2.5.3. Cellcycle

For cell cycle analysis, MG63 cell line was treated with 1mg/mL of zirconia based ceramics, and maintained for 24h in appropriate conditions (37°C, 5% CO_2 and humid atmosphere). Thereafter, cells were harvested, washed in phosphate saline buffer (pH 7.5), fixed in 70% cold ethanol and maintained overnight at -20°C. Each sample was washed in PBS, treated with 1mg/mL RNAse A for 15 minutes and coloured with 100µg/mL propidium iodide by incubation at 37°C for 1 hour. After cells'propidium iodide staining the events acquisition was done using Epics Beckman Coulter flow cytometer. Data were analyzed using FlowJo software and expressed as fractions of cells in different cell cycle phases [9].

3. Results and discussion

Figure 2 shows the XRD of pre-sintered specimens at 1100°C for 2h, where the rapid cooling prevented tetragonal to monoclinic phase transformation for 2.5ZY and 3ZY specimens, except for 2ZY, where the monoclinic phase is also present, according to JCPDS 83-0113 (tetragonal) and JCPDS 72-1669 (monoclinic). This is due to lower content of yttrium oxide for 2ZY.

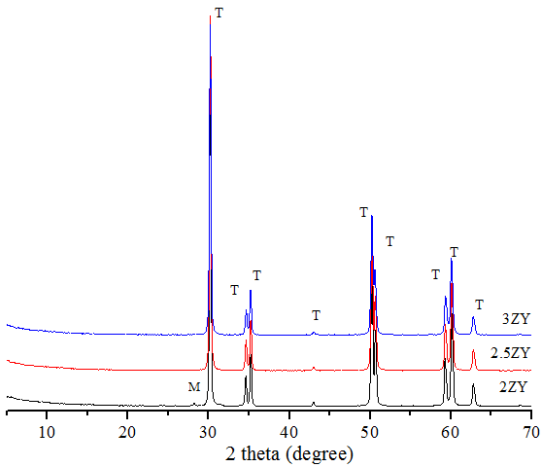

Fig. 2 - XRD of pre-sintered specimens at 1100°C for 2 hours / *Difracția de raze X a probelor presinterizate la temperatura de 1100°C pentru 2 ore (T-tetragonal, M-monoclinic).*

Figure 3 shows the XRD patterns of 2ZY sintered ceramics using thermal histories HIP 1 to 3. The specimens HIPed at 1300°C for 1 hour (Figure 3a) present a mixing of tetragonal and monoclinic phases, according to JCPDS 79-1796 (tetragonal) and JCPDS 37-1484 (monoclinic). Increasing the plateau from 1 hour to 2 hours (Figure 3b) led to the formation of a smaller amount of monoclinic phase, according to JCPDS 83-0113 (tetragonal) and JCPDS 72-1669 (monoclinic). The phase transformation was caused by the low cooling rate of 20°C/min used in HIP treatment and small amount of yttria.

Figure 4 shows the XRD of 2.5ZY specimens, where the tetragonal form is the single crystalline phase, according to JCPDS 83-0113. Analysing the intensity of diffraction peaks, it is noticed that for plateau of 1 hour (Figure 4a), the degree of crystallinity is higher for ceramics sintered by thermal history HIP 3, while for 2 hours (Figure 4b) the degree of crystallinity is higher for ceramics sintered by thermal history HIP 1.

XRD of 3ZY ceramics (Figure 5), indicates the formation of tetragonal phase, according to JCPDS 83-0113. Like the ceramics 2.5ZY, the degree of crystallinity is higher for thermal history HIP 3, plateau 1 hour (Figure 5a) and thermal history HIP 1, with increasing plateau to 2 hours (Figure 5b).

The microstructural study performed on fractured surfaces of pre-sintered samples at 1100°C for 2 hours (Figure 6) reveals a structure with pore size 30 to 40 nm and polyhedral grains of different sizes 50 to 150 nm.

Figure 7 shows the micro structural development 3ZY ceramics hot isostatic pressed at 1300°C for 2 hours. After HIP treatment final dense ceramics were obtained. It is noticed that pores were eliminated after sintering process, particularly for samples that showed pores after pre-sintering thermal treatment at 1100°C. Grains have polyhedral shapes for ceramics sintered after thermal histories HIP 1 and HIP 2, while ceramics sintered after thermal history HIP 3 shows round shaped grains. Grains size ranges from 150 to 300nm. Both, grain size and morphology can be explained by the different obtaining conditions.

The apparent density of the sintered specimens for each thermal history and yttrium oxide molar ratio is given in Table 1. Figure 8 shows the variation of relative density with thermal history and yttrium oxide molar ratio for 2ZY, 2.5ZY and 3ZY. It is noticed that relative densities values are lower for thermal history HIP 1, the highest values being assigned to HIP 2 thermal treatment, where specimens were pre-sintered and then hot isostatic pressed. Relative density is even lower that pre-sintered specimens and this could be caused by the appearance of micro cracks during HIP treatment. When the pre-sintering process was followed by milling and then HIP (HIP 3), specimens presented relative densities values better than thermal history HIP 1.

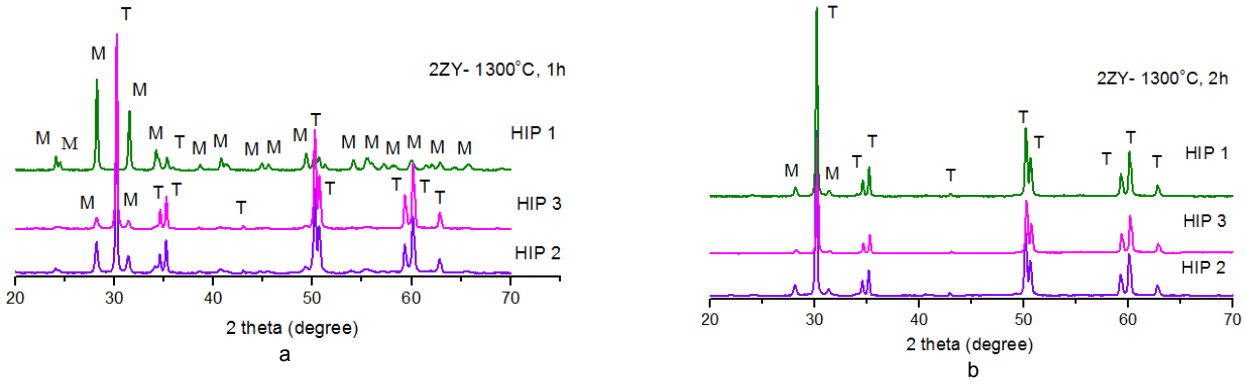

Fig. 3 - XRD of 2ZY HIPed at 1300°C, for a. 1h and b. 2h / *Difracția de raze X pentru 2ZY presate izostatic la cald la temperatura de 1300°C pentru a. 1 oră şi b. 2 ore (T-tetragonal, M-monoclinic).*

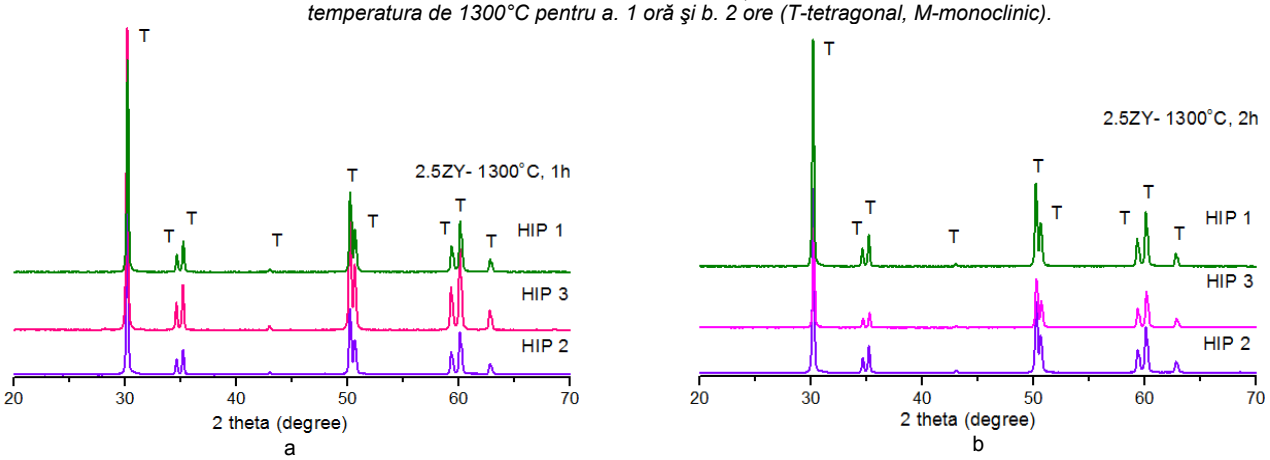

Fig. 4 - XRD of 2.5ZY HIPed at 1300°C, for a. 1h and b. 2h / *Difracția de raze X pentru 2,5ZY presate izostatic la cald la temperatura de 1300°C pentru a. 1 oră şi b. 2 ore (T-tetragonal, M-monoclinic).*

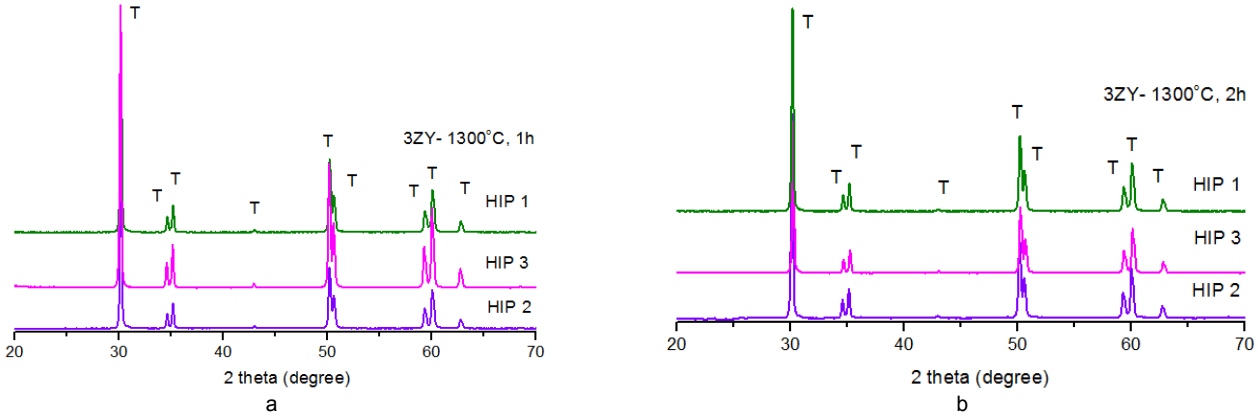

Fig. 5 - XRD of 3ZY HIPed at 1300°C, for a. 1h and b. 2h / *Difracția de raze X pentru 3ZY presate izostatic la cald la temperatura de 1300°C pentru a. 1 oră şi b. 2 ore (T-tetragonal, M-monoclinic).*

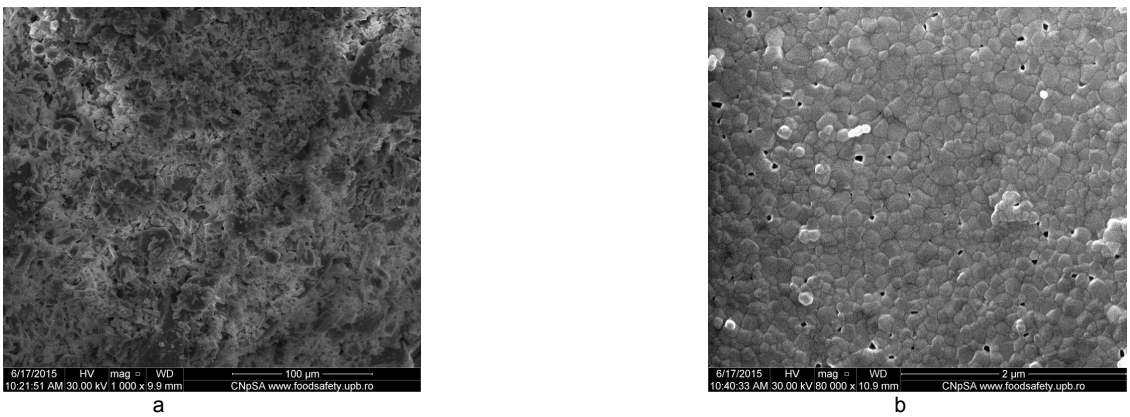

Fig. 6 - SEM images of 2ZY specimens pre-sintered at 1100°C for 2 h / *Imaginile SEM pentru probele 2ZY presinterizate la 1100°C pentru 2 ore.*

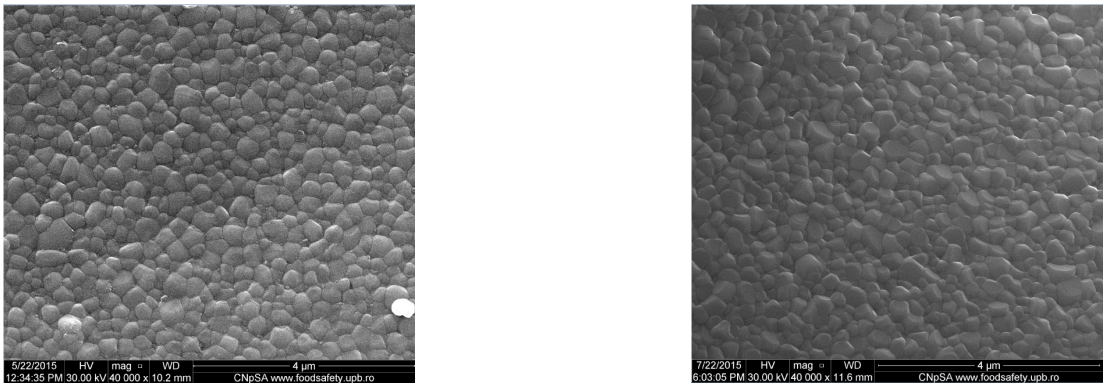

Fig. 7 - SEM images of 3ZY specimens HIPed at 1300°C for 2 hours after thermal history: a. HIP 1; b. HIP 2; c. HIP 3 / *Imaginile SEM pentru probele 3ZY presate izostatic la cald la 1300°C pentru 2 ore, după istoria termică: a. HIP 1; b. HIP 2; c. HIP 3.*

Table 1

Apparent densities values / *Valorile densității aparente (g/cm^3)*

Sintering conditions	Plateau	Y_2O_3 content, mol%		
		2	2.5	3
Pre-sintering	2 hours	5.5	5.43	5.53
HIP 1	1 hour	5.7	5.12	5.17
	2 hours	5.21	5.14	5.22
HIP 2	1 hour	5.98	5.75	5.74
	2 hours	6.09	5.92	6.09
HIP 3	1 hour	5.9	5.98	5.82
	2 hours	5.84	5.66	5.64

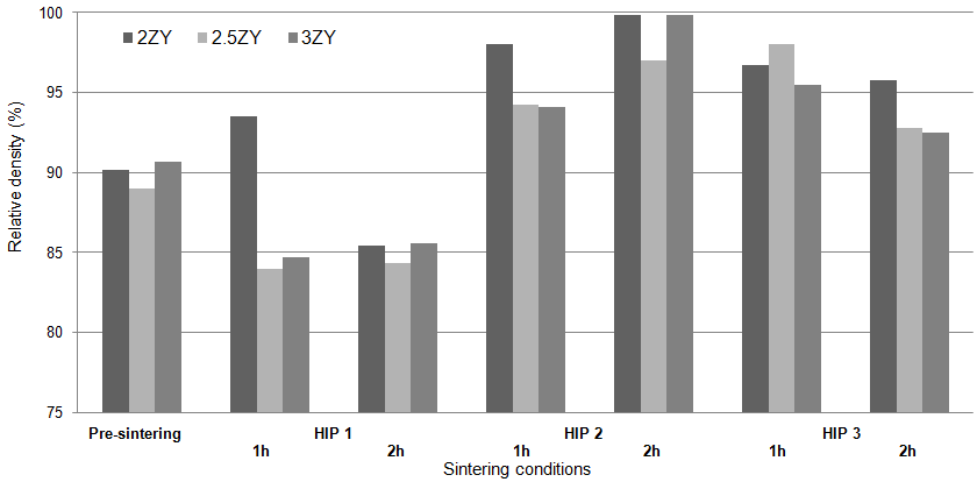

Fig. 8 - Variation of density with thermal history and yttrium oxide molar ratio / *Variația densității relative cu istoria termică şi raportul molar al oxidului de ytriu.*

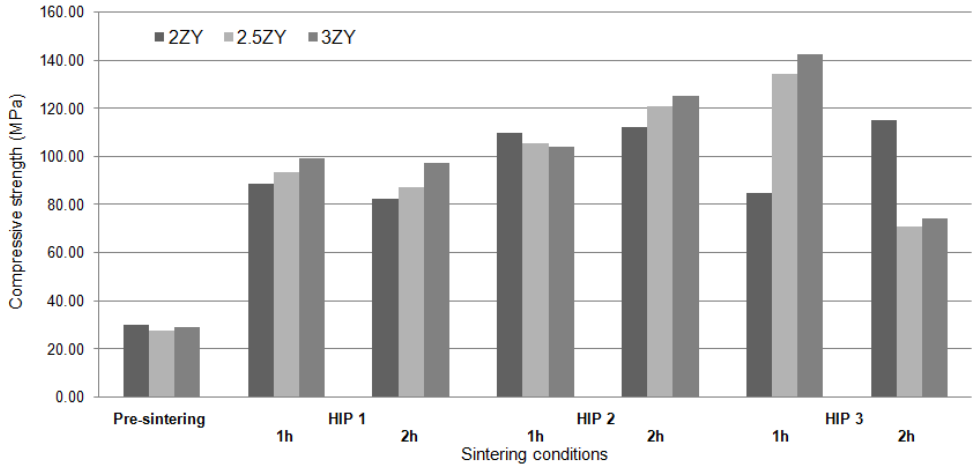

Fig. 9 - Variation of compressive strength with thermal history and yttrium oxide molar ratio / *Variația rezistenței la compresiune cu istoria termică şi raportul molar al oxidului de ytriu.*

Compressive strength is plotted in Figure 9 as a function of sintering conditions and yttrium oxide molar ratio. First, it is noticed that values obtained for specimens that were presureless sintered at 1100°C for 2 hours, show, as expected, the lowest values of compressive strength (<30 MPa). When HIP treatment was applied, compressive strength increased considerably, to at least 70 MPa. Also, mechanical properties were improved by the pre-sintering process, even reaching values of 142 MPa, for 3ZY ceramics sintered after thermal history HIP 3. In general, the best values (aprox. 100 MPa) are for specimens that were pre-sintered and then hot isostatic pressed.

Modulus of elasticity was also determined, the variation of this characteristics with thermal history and yttrium oxide molar ratio being plotted in Figure 10. Ceramics that were hot isostatic pressed presented elastic behaviour, with relative Young's modulus values between 100-319% (2ZY), 100-306% (2.5ZY) and 100-292% (3ZY), where the reference value was for the pre-sintered samples. It is noticed that the lowest elastic behaviour is assigned to thermal treatment HIP 3 and the most pronounced elastic behaviour is for HIP 2.

The treatment of MG63 cells with 1mg/mL zirconia based ceramics proved not to be cytotoxic. Even if the used concentration was quite high (1mg/ml), the contrast phase microscopy has revealed unaltered morphology similar to that observed in untreated cell culture (Figure 11 left). Furthermore, it was observed that both the treated and the untreated cell cultures contain dividing cells without dead (propidium positive) cells (Figure 11 right). Taking into account that the cells maintain their integrity and zirconia based ceramics do not produce cellular lysis or secondary changes, these materials can be included in bioinert biomaterials group.

The good biocompatibility of zirconia ceramics was also sustained by cell cycle evaluation. This proved that the obtained materials do not interfere with the proliferative state of the used mammalian cell line (Figure 12).

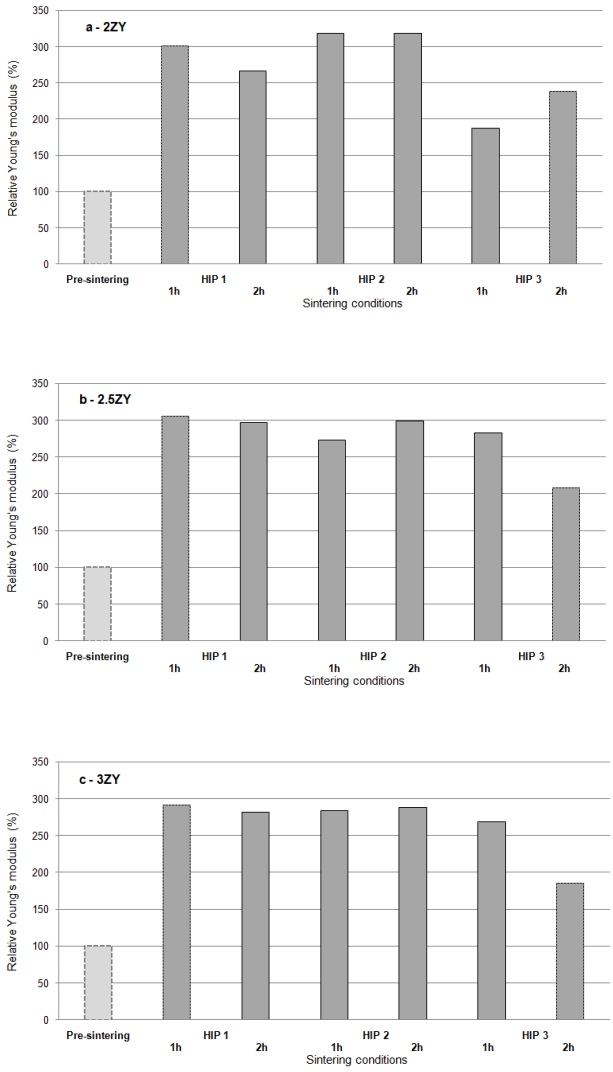

Fig. 10 - Variation of relative Young's modulus with thermal history and yttrium oxide molar ratio for: a. 2ZY, b. 2.5ZY and c. 3ZY / *Variația modulului lui Young relativ cu istoria termică şi proporția oxidului de ytriu, pentru: a. 2ZY, b. 2.5ZY şi c. 3ZY.*

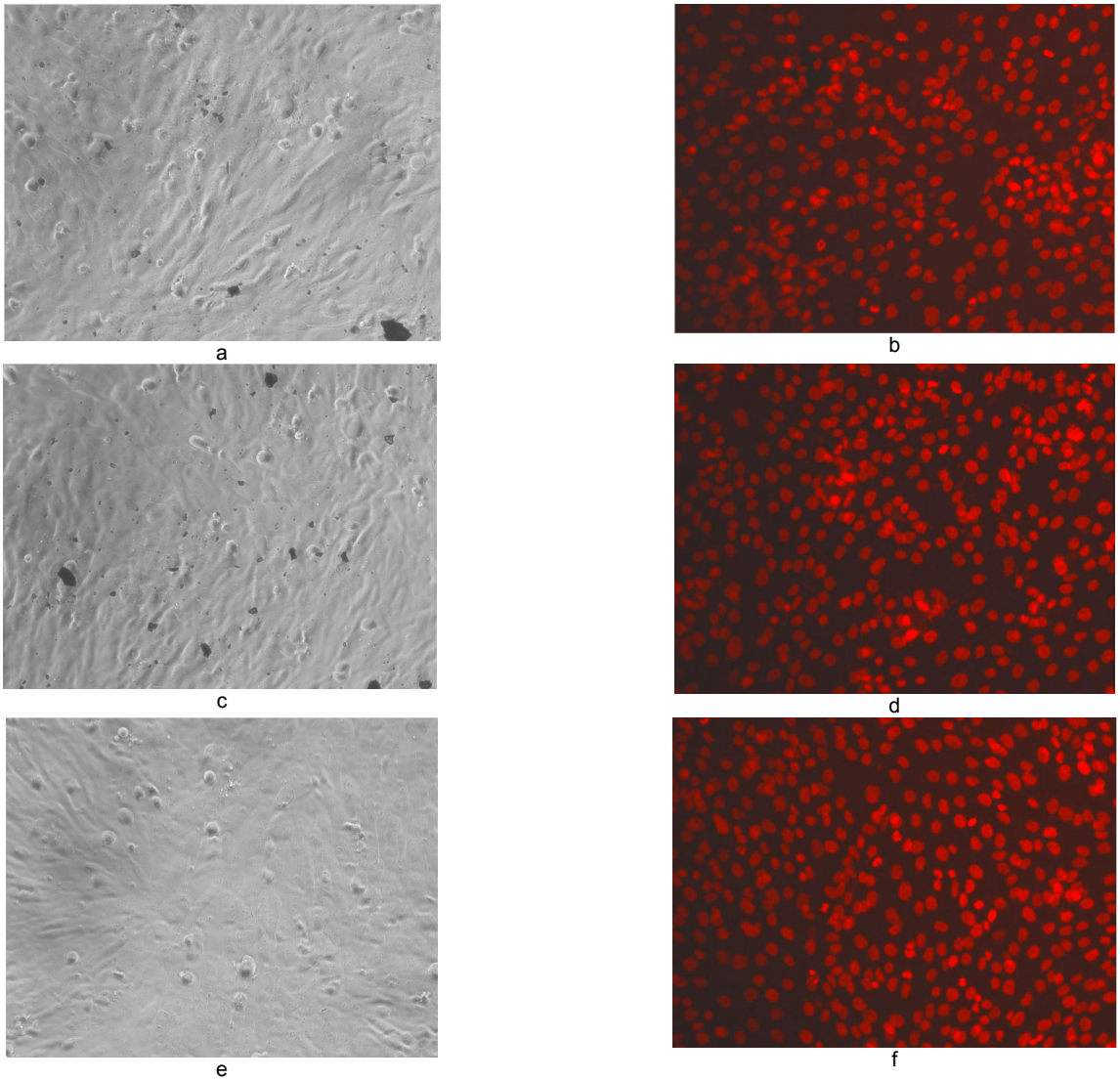

Fig. 11 - Contrast phase microscopy (left) and fluorescence microscopy images of osteosarcoma cells grown in the presence of 3ZY ceramics HIPed using thermal history 1 / *Microscopia în contrast de fază (stânga) şi microscopia de fluorescență a celulelor osteosarcom cultivate în prezența ceramicilor 3ZY presate izostatic la cald după istoria termică 1: a., b. 1300°C-1h; c., d. 1300°C-2h, e., f. MG63, 200x.*

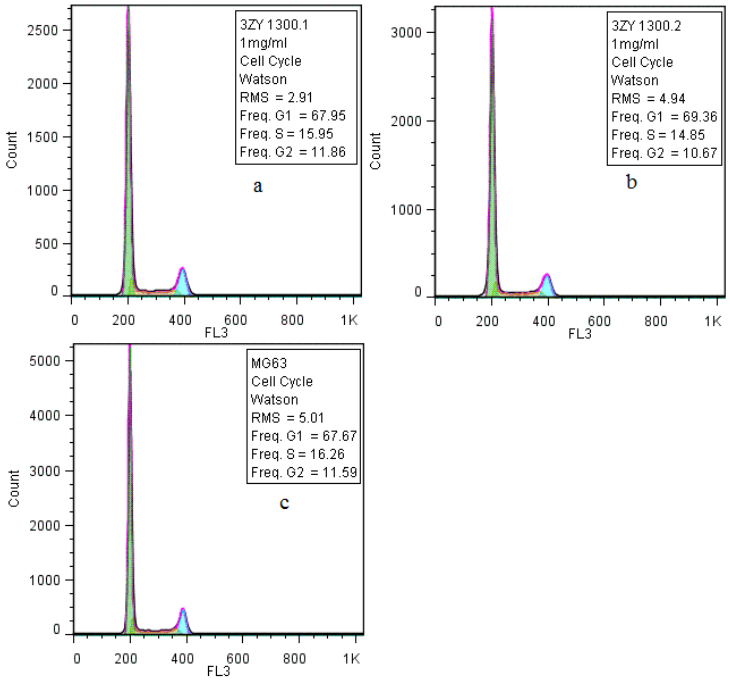

Fig. 12 - Analysis of cell cycle phases in MG63 line treated with 1mg/mL zirconia nanostructures HIPed using thermal history 1 / *Analiza fazelor ciclului celular în linia MG63 tratată cu 1mg/mL nanostructuri de zirconă presate izostatic la cald după istoria termică 1, la: a. 1300°C-1h, b. 1300°C-2h, c. MG63.*

4. Conclusions

The main purpose of this paper is to determine the influence of thermal history and yttrium oxide molar ratio on densification, mechanical properties and biocompatibility of zirconia ceramics.

The following can be concluded:

- ceramics 2ZY sintered using thermal histories HIP 1 to 3 presented a mixing of tetragonal and monoclinic phases, while for 2.5ZY and 3ZY, the tetragonal form is the single crystalline phase;
- pre-sintered specimens at 1100°C, for 2 hours presented a microstructure with pore size 30 to 40 nm, while for hot isostatic pressed ceramics, it is noticed the absence of pores;
- density values were lower for thermal history HIP 1, while the highest values were obtained for thermal history HIP 2, where specimens were pre-sintered and then hot isostatic pressed;
- mechanical properties were improved by the pre-sintering process, best values being obtained for 3ZY specimens sintered after thermal history HIP 2 (pre-sintering followed by HIP);
- the lowest relative Young's modulus values were for HIP 3, while the highest values were assigned to HIP 2;
- all ceramics proved a good biocompatibility, allowing the normal development of MG63 cells in vitro.

The results reported in this work showed that ZrO_2-Y_2O_3 dental ceramics manufactured by hot isostatic pressing, presented compressive strength and a good biocompatibility with human cells.

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