Rheology and porosity effect on the proliferation of pre-osteoblast on zirconia ceramics

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Abstract. It has been studied $ZrO_2(Me_xO_y)$ based porous ceramics, obtained from the powders consisting of hollow spherical particles. It was shown that the structure is represented as a cellular carcass with a bimodal porosity, formed of a large pore close to a spherical shape and the pores that were not filled with the powder particles during the compaction. For such ceramics the increase of pore volume is accompanied by an increase in strain in an elastic area. It was also shown that the porous ZrO_2 ceramics had no acute or chronic cytotoxicity. At the same time, ceramics possess the osteoconductive properties: adhesion support, spreading, proliferation and osteogenic differentiation of MSCs.

1. Introduction

Porous ceramic materials have been successfully used in various fields, including as heat-insulating building materials, because they are durable, corrosion resistant and they possess stable thermal features [1-6]. Porous ceramics are also a promising material for medical use in the field of traumatology and orthopedics for critical sized bone defect recovery. Thus porous ceramics can act both as osteoplastic material or as 3D scaffold for tissue engineered bone equivalent modeling [7].

Ceramics based on partially stabilized zirconium are the most interesting among the variety of ceramic materials due to their inherent high fracture toughness because of their inherent transformational conversion. It is known that the characteristics are determined by the quality of source ceramic powder (particle shape, particle size distribution), the conditions of compacting and sintering modes and any features that are presented in each phase, and how these phases, including pores, are arranged in relation to each other. The most important factor in the successful application of materials is understanding the features of a structure emerging in them on their behavior under mechanical impact.

The aim of a paper is to examine the pore structure of $ZrO_2(Me_xO_y)$ ceramics and its biocompatibility with multipotent mesenchymal stromal cells (MSCs) *in vitro* assays.

2. Materials and methods

The materials for the study were ceramics obtained from powders of $ZrO_2(MgO)$, $ZrO_2(Y_2O_3)$, liquid-phase decomposition of precursors synthesized in high-frequency discharge plasma (the plasma chemistry method). Porous ceramic $ZrO_2(MgO)$, $ZrO_2(Y_2O_3)$ powder was prepared by pressing and subsequent sintering of compacts homologous temperatures ranging from 0.63 to 0.56 during the isothermal holding duration of 1 to 5 hours. The porosity of ceramics $ZrO_2(MgO)$, $ZrO_2(Y_2O_3)$ ranged

from 15 to ≈ 45% and ≈ 30 to 80%, respectively. X-ray studies were carried out on a diffractometer with filtered CuKα radiation. The studies on the ceramic structure were carried out on the scanning electron microscope (SEM) Philips SEM 515.

To assess the biocompatibility of porous ceramics, the adipose-derived MSCs have been used. MSCs were isolated by enzymatic method and cultured in DMEM:F12 supplemented with 2 mM *L*-glutamine and 10% FBS ("Sigma", USA) and incubated with use of integrated continuous live cell imaging and analysis platform Cell IQ® v2 MLF ("CM Technologies"). Third passage MSCs have been used for experiments. Preliminarily MSC culture compliance with minimal criteria was made for phenotype (flow cytometry) and differentiation potential (differentiation assays for adipocytes and osteoblasts) [8]. Prior to seeding over the implants, cell viability in suspension was assessed by Trypan blue staining. [9] To assess the cytotoxicity of the implants and the viability of cultured MSCs over their surfaces, cell combined double staining with fluorescein diacetate (FDA) and propidium iodide (PI) 24h after inoculation and 7 days after culturing has been made [10]. Assessment of cytotoxicity was performed using an inverted fluorescent microscope Axio Observer A1 ("Carl Zeiss", Germany). To further biocompatibility assessment, the MSC osteogenic differentiation assay was performed according to standard protocols [11]. MSCs were cultured in implants or over its surface for 14 days, followed by detection of alkaline phosphatase activity using the BCIP/NBT substrate ("Sigma", USA) [12].

3. Results and discussion

3.1. Powders

Figure 1. (a) represents the SEM-picture of ZrO_2 powder (3 mol.% Y_2O_3), synthesized by the method of plasma chemistry and particle size distribution of the powder size. ZrO_2 powders (3 mol.% MgO) and ZrO_2(3 mol.% Y_2O_3) practically have no difference in morphological structure and they consist of hollow particles of a spherical shape and a large number of units having no regular form. The average particle size of the spherical powders ZrO_2(MgO), ZrO_2(Y_2O_3) was 1.8 and 1.5 microns, respectively.

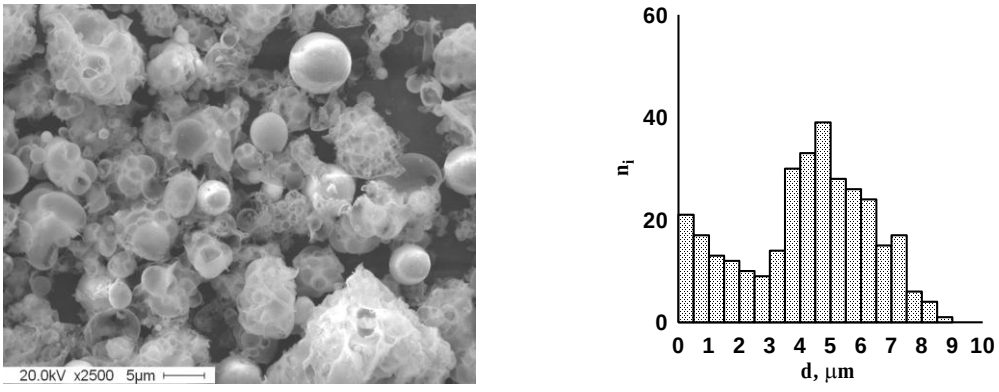

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Figure 1. SEM - picture of ZrO_2 powder (Y_2O_3), synthesized by the method of plasma chemistry (a) and particle size distribution of ZrO_2 powder (Y_2O_3) size (b)

The phase composition of ZrO_2 powder (Y_2O_3) is presented by tetragonal and monoclinic ZrO_2. In the powder ZrO_2(MgO) the cubic, tetragonal and monoclinic phases of ZrO_2 were present. The rate of tetragonal ZrO_2 powder ZrO_2(Y_2O_3) was about 95%, and ZrO_2 in the cubic phase ZrO_2 powder (MgO)

- 75%. The average size of the coherent scattering regions (SCR) tetragonal ZrO_2 in ZrO_2 powder (Y_2O_3) was 20 nm, and the monoclinic modification - 50 nm. The average size of cubic modification SCR of ZrO_2 in ZrO_2 powder (MgO) was 20 nm, monoclinic ZrO_2 - 30 nm, in the tetragonal phase - 15 nm.

3.2. Sintered ceramics

Figure 2 represents the SEM - picture of ZrO_2 ceramics structure (Y_2O_3) and pore size distribution. ZrO_2 ceramics structure (MgO), $ZrO_2(Y_2O_3)$ were represented as a cellular frame. Cells had a nearly spherical shape. The cell size exceeded by many times the thickness of the walls, which was represented as a single ZrO_2 layer stacking grain.

Pore size distribution was bimodal. The first maximum pore was formed by interparticle pores that were not filled with powder particles during compaction and the second - with the larger pores close to a spherical shape. From the optical microscopy data we have obtained dependences of interparticles pores and larger spherical pores, it is seen that the increase in the volume of pores in the material from ≈ 30 to 80% was achieved by reducing the sintering temperature of the samples and it was accompanied by an increase in the average size of large pores from 2 to 6 microns. Changing the porosity of the material had practically no influence on the average size of interparticles pores, the average size of which was 0.5 microns. It can be assumed that the presence of large pores close to a spherical shape in the ceramics is due to the presence of hollow spherical particles in source powders, since their average size is commensurate with an average size of presented large pores in the sintered material.

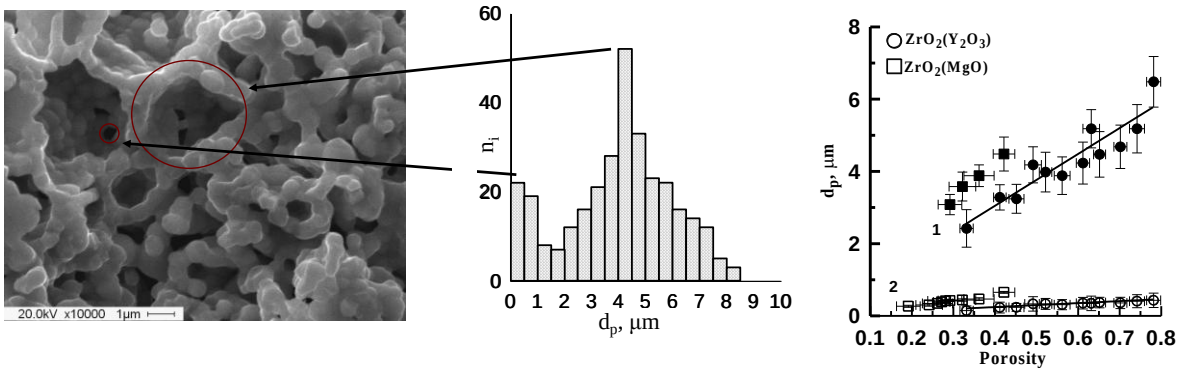

Figure 2. SEM-Picture of $ZrO_2(Y_2O_3)$ ceramics structure, the characteristic pore size distribution of $ZrO_2(MgO)$ ceramics with a porosity of ≈ 40% (a) and the dependence of the average pore size vs. porosity of ZrO_2 ceramics (b). 1) - the average size of large pores spherical-like shape; 2) - the average size of interparticle pores.

From the data presented in Fig. 2 (b) dependences of interparticles pores and larger spherical pores from porosity in ceramics $ZrO_2(MgO)$ and $ZrO_2(Y_2O_3)$ it is seen that the increase in the volume of pores in the material from ≈ 30 to 80 % was achieved by reducing the sintering temperature of the samples and it was accompanied by an increase in the average size of large pores from 2 to 6 microns.

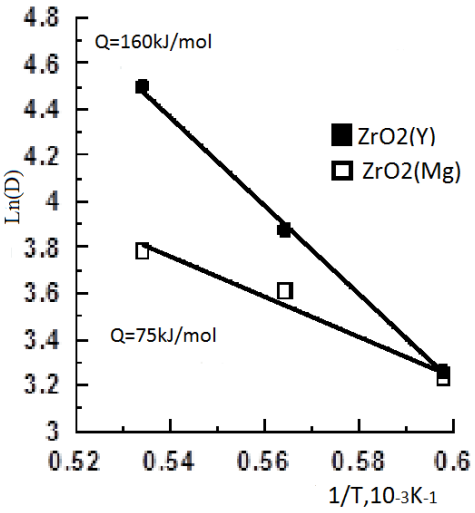

Figure 3. The activation energy calculated from dependence of the crystallites size vs sintering temperature of porous ceramics ZrO_2

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In this study we have determined the activation energy of the crystallites growth for $ZrO_2(Y_2O_3)$ and $ZrO_2(MgO)$ ceramics, Fig.3. It was obtained according to re-plotting of crystallite sizes with increasing sintering temperature, fig.6. Activation energy for growth of crystallites of $ZrO_2(Y_2O_3)$ was 160 kJ/mol, for system $ZrO_2(MgO)$ – 75 kJ/mol, fig. 6, these values are well agree with literature data [13], suggest that the predominant mechanism in the sintering $ZrO_2(MgO)$ is the surface diffusion and for the system ZrO_2 (Y_2O_3) is a bulk diffusion.

3.3. Biocompatibility: cytotoxicity and osteogenic differentiation of MSCs

Cultured adipose tissue-derived MSCs used for a preliminary assessment of the biocompatibility of porous ceramic implants had the capacity to differentiate into adipogenic and osteogenic directions and had the following phenotype: CD73+ CD90+ CD105+ and CD34- CD45-. Combined staining of FDA/PI cells cultured on the surface or in the implants showed no cytotoxicity of porous ZrO2 ceramics (Fig. 4). The results of evaluation of the viability of cells in suspension with use of Trypan blue staining before seeding and 24h after culturing on implants by staining with FDA/PI revealed similar values (suspension, Trypan blue – 96.42 ± 1.8% viable cells; implant, FDA/PI – 93.78 ± 2.15%). Microscopic observation showed that MSCs 24h after seeding adhered to the surface of the implant and generated intensive and uniform green FDA stain in the absence of red PI stain, which indicates a high metabolic activity of the cells and the integrity of their membrane. At the same time the cells had different spreading degree due to the rough surface of the implant through its physical structure (the presence of pores and composition of hollow particles of a spherical shape and a large number of units having no regular form). Cell viability after 7 days of culture with porous ZrO2 ceramic implants was 92.56 ± 1.44%, which is comparable to cell viability before seeding and after 24h culturing with implants (difference not statistically significant). Moreover, after 7 days of MSC culturing on the surface of porous ZrO_2 ceramics, it should be noted the presence of cell clusters due to their proliferation. Thus, porous ZrO_2 ceramic implants do not have the acute and chronic cytotoxicity. Detection of alkaline phosphatase activity with use of BCIP/NBT substrate showed that cultured MSCs on the porous surface of ZrO_2 ceramic implant retain their ability for osteogenic differentiation (Fig. 5). Based on the results of the osteogenic differentiation of MSCs we can conclude that porous ZrO_2 ceramic implants possess osteoconductive properties.

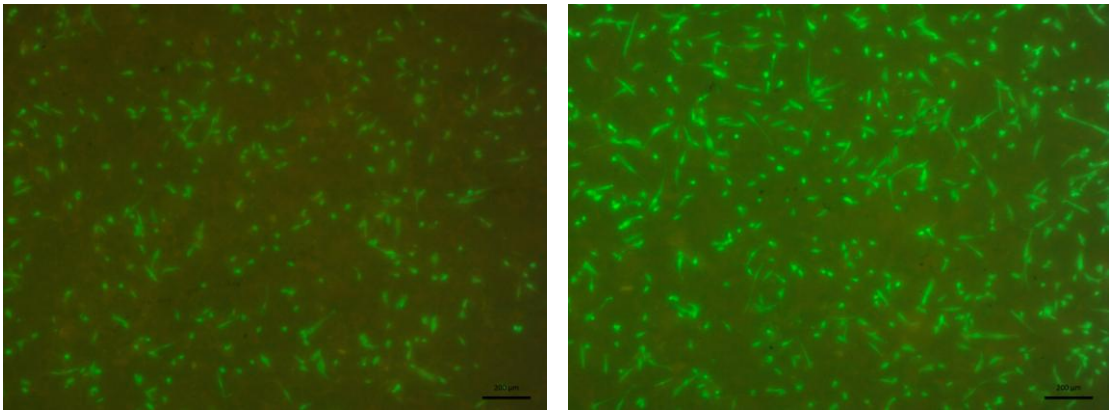

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Figure 4. Viability assessment of MSCs cultured on the porous surface of the ZrO2 ceramic implants for 24h (a) and for 7 days (b). Combined FDA/PI stain.

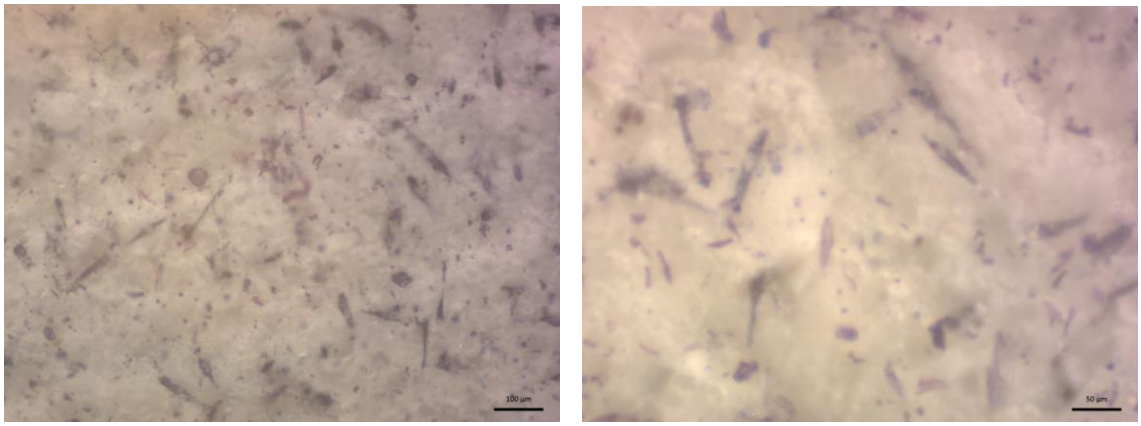

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Figure 5. Detection of alkaline phosphatase activity after osteogenic differentiation of MSCs cultured on the porous ZrO2 ceramic implants. BCIP/NBT stain.

4. Conclusions

It was shown that the structure of $ZrO_2(Me_xO_y)$ ceramics, obtained from the powders consisting of hollow spherical particles with a porosity more 30 % is represented as a cellular carcass with a bimodal porosity, formed of a large pore close to a spherical shape and the pores that were not filled with the powder particles during the compaction.

It was found that in the range of sintering temperatures 0.56-0.63 ceramic $ZrO_2(MgO)$ activation energy of crystallite growth of 75 kJ/mol, which corresponds to the surface diffusion, and for ceramic $ZrO_2(Y_2O_3)$, 160 kJ/mole, which corresponds to the bulk diffusion.

It was also shown that the porous ZrO_2 ceramics had no acute or chronic cytotoxicity. At the same time, the porous ZrO_2 ceramics possess the osteoconductive properties: adhesion support, spreading, proliferation and osteogenic differentiation of MSCs.

Acknowledgments
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