Porous Composite Materials ZrO_2(MgO)-MgO for Osteoimplantology

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Abstract. The pore structure and phase composition of ceramic composite material ZrO_2(Mg)-MgO at different sintering temperatures were studied. The main mechanical characteristics of the material were determined and it was shown that they are close to the characteristics of natural bone tissues. It was shown that material structure has a positive effect on the pre-osteoblast cells proliferation. In-vitro studies of pre-osteoblast cells, cultivation on material surface showed a good cell adhesion, proliferation and differentiation of MMSC by osteogenic type.

INTRODUCTION

Applications of ceramics as osteoreplacement material attract a special interest now. The most actively developed studies in this area are investigations of zirconia ceramic (ZrO_2) included in ISO register as a material for bone replacement. Ceramics based on zirconia stabilized with magnesium oxide (MgO) besides with absence of chemically interaction with body tissues and resistant to most ways of sterilization, like γ-irradiation or steam autoclave treatment has advantages in comparison with other osteoreplacement materials. Moreover, magnesium is involved in protein synthesis and DNA processes, stabilization of DNA molecules, RNA and ribosomes.

Nevertheless, as a material for replacement, zirconia based ceramics has some disadvantages, such as high elasticity, low limiting strain and low resistance to crack propagation in comparison with bone tissue. Solution to the problem of biomechanical compatibility of zirconia-based osteoimplants may be creation of a ZrO_2(MgO)-MgO composite ceramic material [1].

However, in literature there are few data about this system with a porosity close to inorganic bone matrix structure. Therefore, the aim of this paper is to study sintered ZrO_2(Mg)-MgO ceramics and investigate its biological properties for possibility of using in osteoimplantology.

MATERIALS AND EXPERIMENTAL PROCEDURE

The composite ceramic materials based on the mechanical mixtures of powders of zirconia stabilized with 3 mol. % magnesium oxide (ZrO_2(MgO) and magnesium oxide (MgO) in various concentrations, obtained by uniaxial compaction at 180 MPa followed by sintering at 1600°C with isothermal exposure for one hour were

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studied. For obtaining approximately 45% of porosity to the mixtures 50 vol. % of ultra-high molecular weight polyethylene (UHMWPE) particles with mean size 100 μm were added, which were removed during first sintering stage at 300°C for one hour. In the result 43–49% porosity was obtained.

X-ray analysis of ceramic materials was carried out using diffractometer with CuK_α radiation in angle interval 10°–115°, with step 0.05° and 5 s of exposure. The structure of the ceramics was studied by using scanning electron microscope TescanVega 3, its mechanical properties were studied on test machine Devotrans GP.

To evaluate the biocompatibility of porous ceramic materials we used multipotent mesenchymal stem cells (MMSC) which had a typical fibroblastoid morphology, demonstrated the ability differentiate in adipogenic and osteogenic directions and satisfied the minimum criteria for multipotent mesenchymal stromal cells. MMSC were extracted from biopsies of subcutaneous fatty tissue by enzymatic method. The obtained cells were cultured in growth medium composition: DMEM: F12, 10% fetal bovine serum (FBS), 1 ng/ml basic fibroblast growth factor (bFGF), 2 mM L-glutamine (Sigma-Aldrich, USA), 100 Uits/ml penicillin and 100 ug/ml streptomycin (PAA, Austria) and multigas incubation C210 (Binder, Germany) at 37°C, 5% O_2 and 5% CO_2. The medium was changed every 3 days. To evaluate the cytotoxicity of porous ceramics samples and to determine the viability of cells cultured on ceramic surface fluorescein diacetate (FDA, "Life Technologies", USA) and propidium iodide (PI, "Sigma", USA) tests after 24 hours and 7 days after inoculation were used.

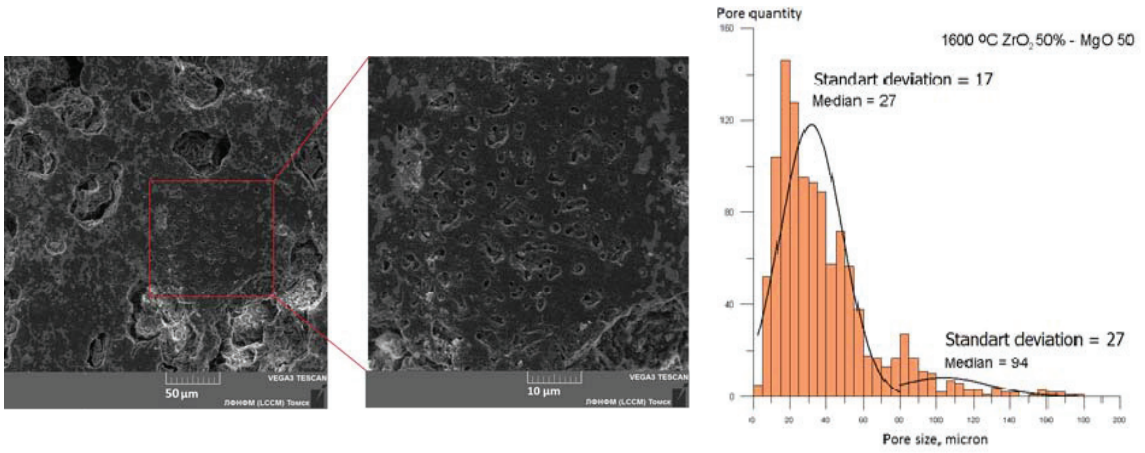
To determine the ability of MMSC to directed osteogenic differentiation during their cultivating on the surface of porous ceramic materials detection of first marker of osteogenic differentiation, alkaline phosphatase, was performed. For this purpose, we used a colorimetric method based on substrate BCIP/NBT: MMSC were seeded on the surface of ceramic samples and cultured for 14 days, then the cells were fixed for 10 min in 4% paraformaldehyde, 0.5 ml of BCIP/NBT, and incubated at room temperature for 20 min, with following microscopy [2, 3].

RESULTS AND DISCUSSION

Figure 1 shows the polished surface of the material and pore size distribution. As one can see, the porosity of two types was formed: with a mean size of 94 μm, formed due to UHMWPE particles and with a mean size 27 μm. Depending on the composition the average pore size varied slightly within the range of 4 microns. Obviously, large pores are communicated with each other and may contribute to MMSC cell adhesion at in vitro studies [4].

Table 1 shows the results of mechanical properties and the mean pore size with different content of magnesia. As one can see, the maximal strength corresponds to MgO, 33 MPa and with increasing of ZrO_2(Mg) content tensile strength reduce to 18 MPa.

Figure 2a shows integral intensity of phases vs. MgO concentration. The intensity of ZrO_2 cubic phase peaks decreases with increasing in magnesia concentration, but this dependence is nonlinear, which can be explained by the different absorption coefficient of components.



(a) (b)

FIGURE 1. (a) Surface of ceramic and (b) pore size distribution in 50% ZrO_2(MgO)–50% MgO ceramic

TABLE 1. The compressive strength and the mean sizes of porosity

ZrO_2, %	σ, MPa	*E*, GPa	Mean pore size of I-type, μm; standard deviation	Mean pore size of II-type, μm; standard deviation
100	18.2	1.6	29; 19	110; SD = 31
75	21	1.8	30; 23	104; SD = 21
50	28.5	2.4	27; 17	94; SD = 27
25	32.5	2.3	26; 17	101; SD = 30
0	33.1	3	28; 20	105; SD = 27

From X-ray patterns the sizes of coherent diffraction domains (CDD) of phases and their lattice microdistortions were calculated. It was found that with the increase in MgO concentration the mean size of ZrO_2 cubic phase crystallites increased from 400 to 600 Å, while the microdistortions decreased from 0.025 to 0.01. MgO crystallites size almost did not change and its values were approximately 60 nm, but lattice microdistortions decreased from 0.009 to 0.003. On the fracture surface the mean size of CDD of zirconia and magnesia much larger, this may stipulate intercrystallite type fracture [5, 6].

Figure 2b shows the microstresses calculated from microdistortions vs. macrostresses obtained from mechanical tests. As one can see ceramics strength directly depends on microstresses, this means that grain boundaries are very important in mechanical properties formation [7].

Determining MMSC ability to aimed osteogenic differentiation during their cultivating on the porous ceramic samples was performed by alkaline phosphatase detection, which is a first marker of osteogenic differentiation. Figure 3a shows undifferentiated MMSC (dark areas in the figure) in marker-medium, which do not express or weakly express alkaline phosphatase and give only background staining. After 7 days of culturing various degrees of cells propagation with the highest activity on the composition with 25% of MgO concentration were observed. Figure 3b shows the results after 14 days of cultivation in osteoinductive medium: cells differentiate into osteogenic direction, grow and agglomerate and give saturated media staining with colorimetric detection of alkaline phosphatase. The mean cell viability on the ceramic surfaces was about 93%, which is comparable to cell viability before planting. In addition, the presence of cell clusters in the pores should be noted, which may be described by their proliferation [9].

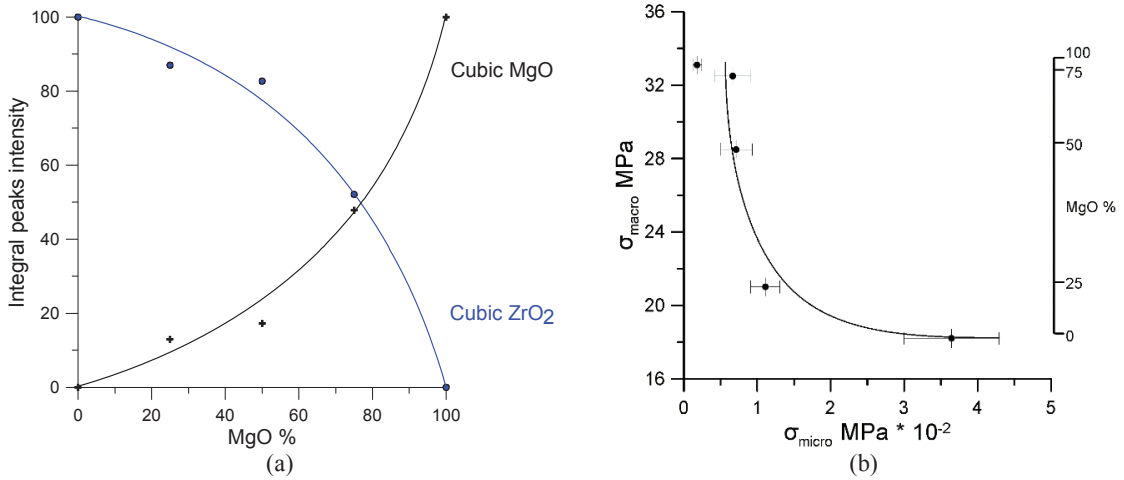
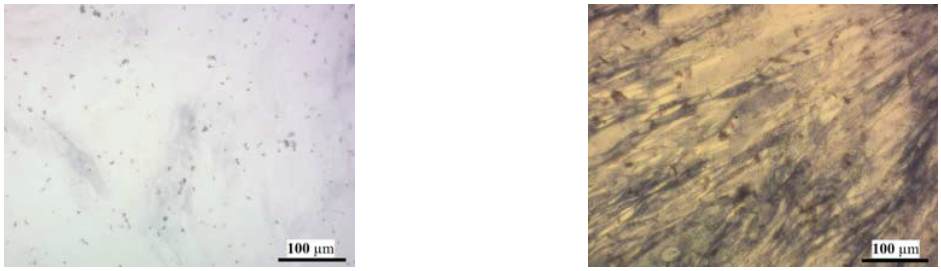


FIGURE 2. (a) Intensity of zirconia cubic phase and MgO of crystal lattice in the composition, (b) dependence of micro- from macrostress of studied ceramics



(a) (b)

FIGURE 3. Detection of MMSC alkaline phosphatase. Transmitted light microscopy. (a) Undifferentiated MMSC, (b) cultured for 14 days MMSC which differentiate into osteogenic direction are painted

CONCLUSION

It was shown that during sintering of porous ceramic the bimodal porosity structures with the mean size 26–30 and 94–110 μm were formed.

Ceramic strength directly depends on microstresses and at high microstresses ceramic has a low strength.

In vitro studies showed that the tested materials are not cytotoxic, cultured MMS cells on the surface of the samples have high viability and osteogenic differentiation ability, and the presence of cell clusters in the pores of the samples may indicate their proliferation.

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The study reported in this article was conducted according to accepted ethical guidelines involving research in humans and/or animals and was approved by an appropriate institution or national research organization. The study is compliant with the ethical standards as currently outlined in the Declaration of Helsinki. All individual participants discussed in this study, or for whom any identifying information or image has been presented, have freely given their informed written consent for such information and/or image to be included in the published article.

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