ZIRCONIA / ALUMINA COMPOSITE FOAMS WITH CALCIUM PHOSPHATE COATING

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Resume

In this study, mechanical properties of calcium phosphate foams were enhanced by zirconia/alumina porous cores prepared by polymer replica technique. This technique was chosen to ensure interconnected pores of optimal size for cell migration and attachment. The porosity of ZA cores (50 – 99%) was controlled by multistep impregnation process, the size of pore windows was 300 – 500 μm. Sintered ZA cores were impregnated by hydroxyapatite or β-tricalcium phosphate slurry to improve bioactivity. The bone like apatite layer was formed on coatings when immersed in a simulated body fluid. Neither of tested materials was cytotoxic. Thus, the composite foam can be potentially used as a permanent substitute of cancellous bone.

Article info

Article history:
Received 16 March 2016
Accepted 29 March 2016
Online 12 June 2016

Keywords:
Scaffold;
Zirconia;
Porosity;
Polymer replica technique.

Available online: http://fstroj.uniza.sk/journal-mi/PDF/2016/06-2016.pdf

ISSN 1335-0803 (print version)
ISSN 1338-6174 (online version)

1. Introduction

A lot of research has been done in the field of bone substitutes up today. Ceramic materials seem to be a reasonable choice for reconstruction of non-healing bone defects due to high biocompatibility, high corrosion resistance in body environment and good compressive strength. The highest chemical similarity to the mineral component of mammalian bones was reported for calcium orthophosphates, such as hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) [1]. Hydroxyapatite has excellent bone bonding ability and bone ingrowth properties [2] however it is extremely weak and brittle mainly in porous form and that is why it cannot be used for load bearing components. Porous structure is nevertheless one of the essential criteria in bone tissue engineering because it facilitates cell migration, vascularization and further integration in the body and it provides a mechanical support to the newly formed bone tissue [3]. It is known that the increasing porosity negatively influence the mechanical resistance of ceramics with exponential dependence [4]. The strongest and the most toughened bioceramics compare to other ceramic materials is undoubtedly tetragonal zirconia (TZP) [5], which is unfortunately nearly bioinert and do not directly bond to bone tissue [6].

It seems reasonable to combine great mechanical properties of zirconia with outstanding biological properties of hydroxyapatite to overcome drawbacks of both materials and prepare composite structure. Various studies [7 - 9] dealt with this approach, however, several problems, such as poor sinterability or decomposition of hydroxyapatite were reported. In the case of zirconia cores coated by hydroxyapatite, the interfacial bonding was problematic.

The purpose of this study was to prepare porous zirconia / alumina composites suitable for bioactive coatings. Alumina can improve the mechanical strength of TZP due to thermal expansion coefficient mismatch between TZP and alumina, resulting in tensile residual stresses in the composite which reduces the critical stress level for tetragonal to monoclinical transformation. Another benefit of alumina is preventing zirconia from unfavourable low temperature degradation in body environment [10] and improving interface bonding between zirconia and bioactive calcium phosphate layer.

Zirconia / alumina foams were prepared by polymer replica technique [11]. This technique was chosen because of its versatility and the possibility to fabricate foam macrostructure that closely resemble the structure of trabecular bone. Slurry technique was chosen for bioactive coatings because it is a simple and inexpensive method resulting in thick (> 20 μm) and microporous structure which ensures long-term clinical stability of implant. The relationship between structure and properties of prepared composites was studied in this paper.

2. Materials and methods

2.1 Preparation and sintering of porous ceramic foams

Ceramic foams were prepared via polymer replica technique. Polymer sponge templates (Bulpren S28089 and S28133, Eurofoam, Czech Republic) cut into blocks of 13×13×10 mm were subsequently immersed into the ceramic slurry. The slurry was prepared from commercial powders Disperal P3 (nanopowder based on boehmite, Condea Chemie, Germany) and HWY-5.5SD (3 mol% yttria stabilized zirconia, Guang Dong Huawang Materials, China). Zirconia powder was bonded by boehmite sol stabilized by 1 M acetic acid. The amount of zirconia powder in the slurry was determined by means of thermal analysis of AlOOH to achieve 2.5, 5 and 10 wt.% of alumina in the structure after sintering. Weight fraction of solid phase in prepared slurries reached 50, 55 and 60 wt.%. The templates were immersed into the slurry, and slurry surplus was removed by application of compressed air. So-prepared foams were dried at 70 °C. The immersion and drying process was repeated until the desired porosity was achieved. The polymeric sponge was subsequently burnt out at 1000 °C for 2 h and pressureless sintered in air at 1550 °C / 2 h. Some of the sintered cores were consequently impregnated by calcium phosphate based slurries with the aim to improve bioactivity. Two commercial powders, hydroxyapatite and β-tricalcium phosphate (both Fluka, Switzerland), were added into Butvar B79 (PVB, Solutia Inc., US; 2 wt. % relative to the powder) dissolved in isopropanol. The slurries, containing 40 wt. % of solid phase, were stirred for 2 hrs. Sintered cores were then immersed into the suspension and the excess slurry was removed by compressed air. This step was repeated twice to achieve a homogenous coating. Composite foams were sintered at 1200 °C in air atmosphere.

2.2 Characterization of slurries and sintered ceramic foams

Thermal analysis of boehmite powder was performed by 6300 Seiko Instruments TG-DTA in a mixture of air and argon in a ratio of 1:1; flow rate 400 ml / min and a temperature increase 2 °C / min. The zeta potential was evaluated between pH 2 and pH 9 by Zetasizer 3000 HS (Malvern Instruments, UK). Rheological properties of sols and slurries were measured by a rotary rheometer Mars II (Haake, Germany) at shear rate 0.5–1000 sec^{-1}. The morphology of sintered foams were observed with the use of digital camera AM4115ZTL (DinoLite, Netherlands) and scanning electron microscopy (XL30, Philips, Netherlands and ZEISS Ultra Plus, Germany). The total porosity was calculated from the volume, the mass and the theoretical density as follows [12]: $P = \frac{\rho_t - \rho_b}{\rho_t} \times 100\%$, where ρ_t is the theoretical density and $\rho_b = \frac{m_b}{V_b}$ is the bulk density,

m_b is the mass of the dry test piece and V_b is the total geometrical volume.

The pore sizes and strut thicknesses were determined using image analysis by ImageJ software (National Institutes of Health, US). The samples were embedded in a resin, cut, ground and polished by standard ceramographic methods. The pore size was estimated on cross-sections of ceramic foams.

2.3 Compressive strength

Mechanical properties of prepared ceramic foams were determined by compression test. High-Precision Electric Actuator Systems 8862 (Instron, US) equipped with 5 kN load cell was used for the loading. The crosshead speed was 0.5 mm/min. The compressive strength was determined from the maximal obtained force.

2.4 Biological properties of prepared foams

Bioactivity of cores and coatings was biochemically tested in a simulated body fluid. The solution was prepared according to the corrected Kokubo recipe [13]. Specimens were immersed into SBF solution at 36.5 °C for 1, 2 and 4 weeks. After removal from the solution and rinsing with deionized water, the samples were dried at room temperature for 24 hrs. The presence of the newly formed apatite layer on the surface was observed by SEM.

Cytotoxicity of materials was tested in vitro using direct contact assay according to EN ISO 10993-5 specifications. Ceramic samples were sterilized in autoclave for 20 min at 121 °C. Human osteosarcoma cells of cell line MG63 (from European Collection of Cell Cultures) were cultivated at 37 °C in 5 % CO_2 in Eagle Minimum Essential Medium (EMEM) with high glucose, 1 % L-Glutamine, 1 % non-essential amino acids (NEAA), 10 % fetal bovine serum, 1 % antibiotics and 0.3 % gentamicin. Cell line was then inoculated into a measured amount of cultivation medium and transferred to the surface of sample with the density of 3 500 cells/cm^2 of a cultivation vessel. After exposition period (8 or 72 hours) samples were washed by PBS twice and cells were fixated and dried by increasing alcohol series. Dried cell-seeded samples were examined by SEM. Tolerance of cells towards the tested material was evaluated by calculating the percentage of adhered cells.

3. Results

3.1 Characterization of suspensions and sintered foams

Thermal analysis

Thermal analysis was carried out to determine the amount of Al_2O_3 after decomposition of commercial boehmite powder. The TG curve (Fig. 1) indicated a total mass loss of 30.5 %. This value was slightly higher than 28% mass loss reported by the manufacturer, probably due to physically adsorbed water.

Zeta potential

Zeta potential, an indicator of the stability of colloidal dispersions, was measured in aqueous dispersions of boehmite. Results showed that sols behaved stably in acidic range (the ζ-potential between pH 2 and pH 6.5 exceeded 30 mV). The highest value (+60 mV) was measured at pH 4. In alkaline environment, dispersions were not electrically stabilized and tended to coagulate (the ζ-potential was between 20 and -15 mV).

In further experiments, suspensions were prepared from the most stable sols with pH adjusted by acetic acid to pH 4.

Viscosity of suspensions

The viscosity of sols was changed during aging. When the aging time increased, the viscosity decreased from 4 mPa·s to 2.5 mPa·s. The sufficient time of aging was 24 h, when the supposed de-agglomeration of powder was finished and sol was stabilised with almost Newtonian rheological behaviour. The aged sol was filled with zirconia in order to prepare zirconia / alumina (ZA) composite

at weight fraction of solid phase 0.5 to 0.6. Viscosity of slurries increased with increasing content of solid phase from 0.3 to 4 Pa·s (at shear rate 5 s^{-1}) and from 0.06 to 0.4 Pa·s (at shear rate 100 s^{-1}). All prepared slurries exhibited slightly thixotropic behaviour which is beneficial for polymer replica technique.

From a technological point of view, the best results for the 45 ppi sized foams were achieved when impregnated by suspension containing 60 wt. % of solid phase. However, this suspension was too viscous for coating of foams with smaller pores. To avoid blocking of open cells ("macropores"), the foams with 60 ppi porosity were coated by suspension containing 50 wt. % of solid phase.

Morphology of sintered ceramic foams

Ceramic foams of different total porosity and pore size were prepared by repeating immersion of PU templates into the ceramic suspension. Morphology parameters of sintered foams such as total porosity, strut thickness, cell size and size of windows between adjacent macrocells (pore size) of prepared foams are summarized in Table 1.

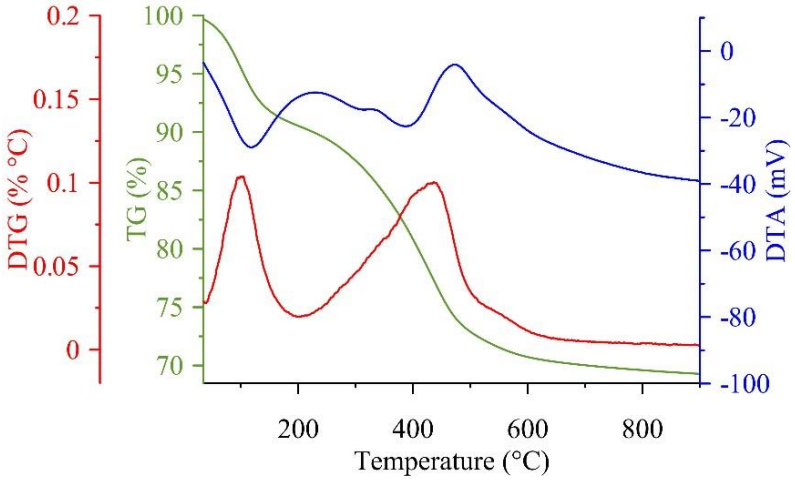

Fig. 1. Thermal analysis of commercial boehmite powder Disperal P3.
(full colour version available online)

Table 1

Porosity, strut thickness, cell size and pore size of ZA foams.

PU porosity	n layers	Porosity (vol. %)	Strut thickness (µm)	Scaffold cell size (µm)	Pore windows size (µm)
45 ppi	1	97 – 99	100 ± 18	1260 ± 420	500 ± 330
45 ppi	2	93 – 94	150 ± 35	1250 ± 390	480 ± 320
45 ppi	3	87 – 90	170 ± 40	1170 ± 380	440 ± 280
45 ppi	4	77 – 81	270 ± 70	990 ± 330	340 ± 240
45 ppi	5	62 – 65	400 ± 130	—	—
45 ppi	6	50 – 55	—	—	—
60 ppi	1	96 – 97	70 ± 17	750 ± 320	380 ± 230
60 ppi	2	91 – 93	100 ± 27	720 ± 240	320 ± 170
60 ppi	3	77 – 83	ca 300 ± 160	—	—
60 ppi	4	59 – 64	ca 340 ± 170	—	—

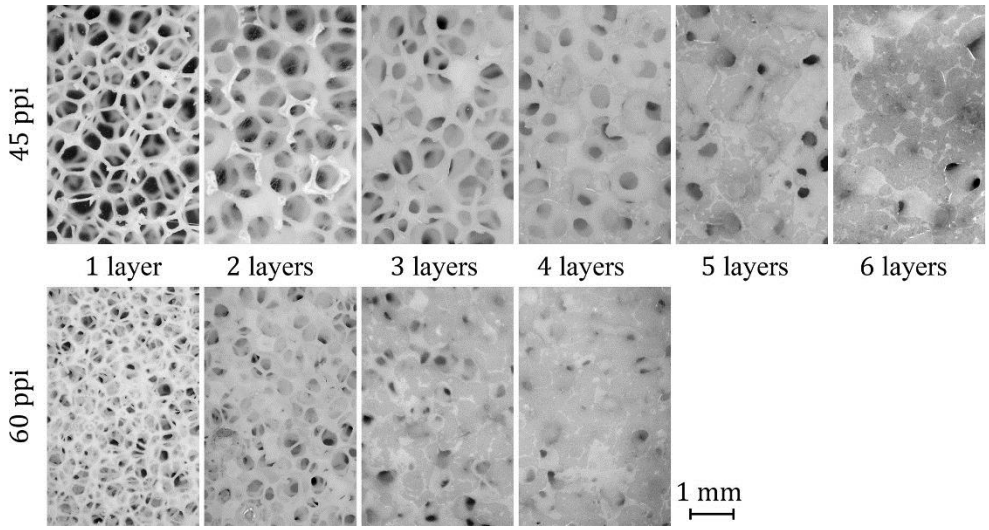

Fig. 2. Thermal analysis of commercial boehmite powder Disperal P3.

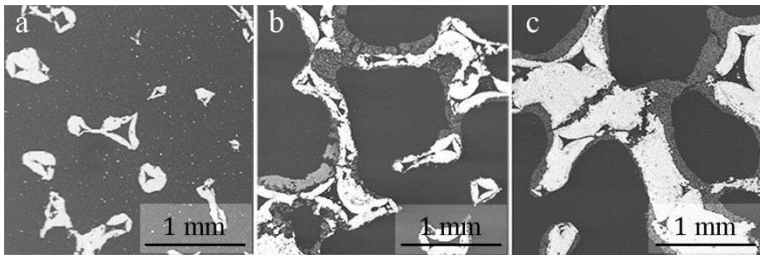

Fig. 3. Cross-sections of bioceramic foams: (a) ZA; (b) ZA coated by HA; (c) ZA coated by β-TCP.

It is evident, that the initial porosity of the polymeric template had a crucial influence on the final foam cell size after sintering. Cell size of ceramic foam corresponded to the cell size of polyurethane foam reduced by shrinkage and by thickness of the strut. With an increasing number of layers, the average thickness of struts grew. The typical reticulated morphology was lost as porosity decreased below 70 % (in the case of larger pores – 45 ppi) and below 80% (for 60 ppi). Overview of macrostructures of prepared foams is shown in the Fig. 2.

Zirconia / alumina substrates with desirable porosity were coated by calcium phosphates in order to improve the biological properties of scaffolds. Foams consisted of 2 layers of ZA (containing 5% Al_2O_3) and CaP coating are shown in Fig. 3. The β-TCP coating firmly adhered to the ZA core. The thickness of both bioactive layers was 20 to 60 μm.

3.2 Mechanical properties

Resistance of some ZA foams to the mechanical loading was characterised by compressive strength. The overview of compressive strength values of ZA foams containing 2.5 to 10 wt. % of alumina is plotted in Fig. 4.

It was experimentally confirmed that strength decreases with increasing porosity. Specimens containing 5 % of alumina exhibited the highest strength, thus this composition was chosen for subsequent biological testing. The measured strength was in accordance with the strength of cancellous bone at comparable porosities. Compressive strength of less porous specimens were not evaluated because all tested samples exhibited strength above the minimum value reported for highly porous cancellous bone [14]. Nevertheless, to ensure sufficient strength to withstand forces generated in the body

it would be convenient to use less porous structures.

3.3 Biological properties

Interaction with SBF

The typical globular morphology of newly formed apatites were locally observable on both HA and TCP coatings after 1 week of immersion in SBF. The areas containing apatite widespread after 2 weeks. The surface of HA and TCP samples was completely covered by a newly formed apatite layer after 4 weeks. The top view of ZA substrate and Ca-phosphates coatings before and after 4 weeks immersion in SBF is reported in Fig. 5. No change was observed on uncoated ZA substrate.

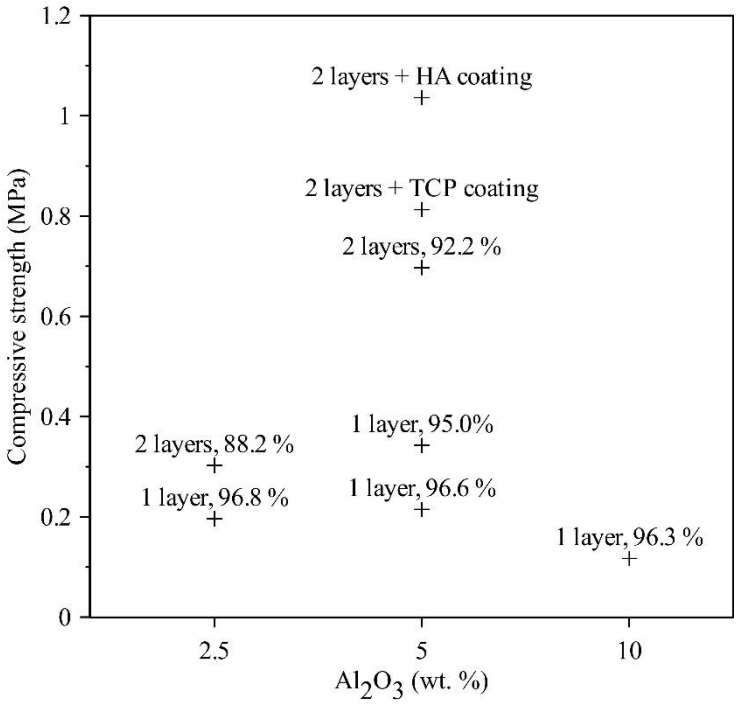

Fig. 4. Compressive strength of porous ZA foams.

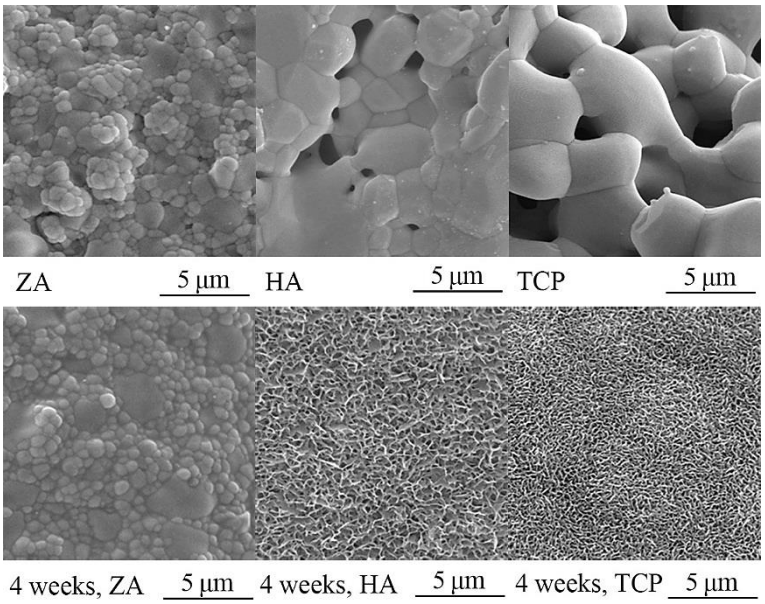

Fig. 5. Microstructure of the ZA substrate and ZA coated by HA a β-TCP before and after immersion in SBF.

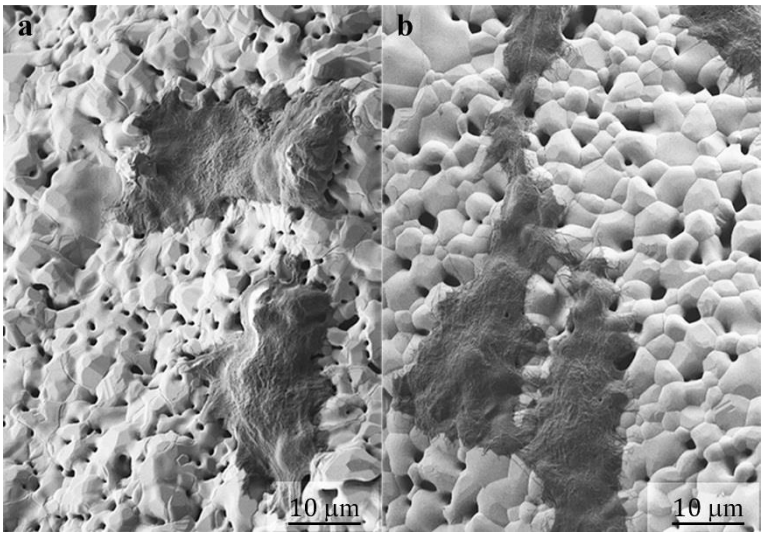

Fig. 6. Cell line MG-63 adhered to the inner struts of: (a) HA; (b) β-TCP.

Cytotoxicity evaluation

Although both zirconia and alumina are consider being bioinert in contact with host tissue, the cytotoxicity of the composite was evaluated by direct contact assay. Direct contact between MG-63 cells and all tested materials did not induce any adverse effect, cells retained characteristic morphology of MG-63 cells (see Fig. 6). The cell density and shape did not differ significantly from that observed for the negative control. Filopodia stretched out from the cells indicated a good adhesion to the bioactive surface of calcium phosphates [15]. The smallest amount of cells (58 %) adhered to the uncoated ZA, 76% of seeded cells adhered to HA layer and the largest number of cells (87%) was observed on the surface of β-TCP coating.

SEM was also used to observe the adhesion and spreading of MG63 cells inside bioactive ceramic foams after 3 days. Micrographs revealed that cells were attached to the walls of struts inside the 3D structure at comparable number as on outer struts. These results suggest that cell migration throughout the 3D structure was possible and that the nutrition and oxygen supply was satisfactory. This result confirmed that all tested materials (ZA, HA, β-TCP) were cytocompatible and could be used in bone tissue engineering applications.

4. Conclusions

Zirconia / alumina based porous foams were fabricated from 3 mol% yttria stabilised zirconia and colloidal boehmite by polymer replica technique. ZA foams of wide range of porosities (50–99 %) were fabricated by multiple impregnation process. Pores were interconnected with dimensions between 300 and 1500 μm. Based on the results of mechanical testing, 5 wt. % content of alumina was chosen as optimum. The compressive strength of prepared ceramic foams was close to the strength of cancellous bone of the same porosity. The bioactivity of the composite foams was improved by calcium phosphate based surface layer. After 4 weeks in SBF, newly precipitated apatite was observed on both calcium phosphates coatings. Neither of tested materials was cytotoxic. MG-63 cells were able to migrate through the 3D porous structure. The reinforced composite foam behaved bioactive so it can be potentially used in bone tissue engineering applications.

Acknowledgements

This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II. The authors would

like to sincerely thank Dr. Zdeněk Chlup for measuring the compressive strength and Institute of complex systems in Nové Hrady for providing biological testing in direct contact assay.

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