Calcium phosphate ceramics having hierarchical pore structure

Marek POTOCZEK*

Rzeszów University of Technology, Faculty of Chemistry, Department of Industrial and Materials Chemistry, 6 Powstańców Warszawy Ave., 35-959 Rzeszów, Poland

ABSTRACT**:** Currently, the most studied materials of porous resorbable ceramics in the field of bone tissue regeneration and as scaffolds in tissue engineering are calcium orthophosphate $Ca_3(PO_4)_2$ and hydroxyapatite $Ca_{10}(PO_4)_5(OH)_2$. In this work, calcium orthophosphate ceramic foams were produced by gel-casting of foams method using agarose as a gelling agent. Foaming was carried out at 60°C, followed by the transformation of the foams from the liquid state to the gelled state by cooling them to 15°C. After the sintering process (T = 1100°C, t = 2 h), the basic physical properties of the foam were determined and morphological observations were made using scanning electron microscopy. The foam exhibited a hierarchical pore structure, i.e., spherical macropores with diameters ranging from 250 to 800 µm, interconnections between macropores (so-called "windows") with diameters in the rage of 30 - 350 µm, and micropores in the ceramic skeleton with diameters ranging from less than 1 to about 3 µm. This structure allows good conditions for bone tissue to grow into the implant.

Keywords: bioceramics, porosity, gel-casting of foams, $Ca_3(PO_4)_2$.

Introduction

Ceramic biomaterials, due to the body's response, are divided into inert biomaterials, active surface biomaterials and resorbable biomaterials. Implantation of resorbable ceramics in areas of tissue defect leads to the induction of biological and chemical processes due to the interaction of the ceramic material and living cells that promote tissue regeneration. Ions released from bioceramics, including phosphorus or calcium play an important role during the regulation of migration and differentiation of osteoblasts during bone formation in vivo [1-4].

In terms of filling the space in the material, ceramic biomaterials are divided as dense and porous materials. Porous materials contain empty spaces called pores. With appropriate conditions including pore sizes and inter-pore connections, the bone tissue growth into the implant is possible which ensures its very good mechanical stability. Porous ceramic biomaterials have found applications as implants for bone tissue reconstruction, drug carriers in targeted therapy and as scaffolds in tissue engineering. Depending on the area of application, porous ceramic biomaterials have to fulfil several requirements in terms of the dimensions of the pores and the dimensions of the connections between them. These dimensions determine the penetration of tissue into the implant and its mineralization, which enables a good and durable connection between the implant and the bone. It is currently accepted that optimal bone tissue ingrowth is demonstrated by implants with pore sizes in the range of 100-500 µm and interconnection pore sizes above 50 µm [5]. Currently, the most studied porous resorbable ceramic materials in the field of bone tissue regeneration and as scaffolds in tissue engineering are calcium orthophosphate [$Ca_3(PO_4)_2$] of the β polymorphic variety (β-TCP) and hydroxyapatite [$Ca_{10}(PO_4)_5(OH)_2$] (HAP) [4-9]. Although, β-TCP and HAP have both biocompatible and osteoconductive properties, β-TCP is more rapidly resorbable indicating that it can interact with hard tissues. This important characteristic makes β-TCP an interesting material for the production of temporary scaffolds that degrade with progressive bone ingrowth thus enabling bone regeneration [9].

*Corresponding author at: Rzeszów University of Technology, Faculty of Chemistry, Department of Industrial and Materials Chemistry, 6 Powstańców Warszawy Ave., 35-959 Rzeszów, Poland;
E-mail: potoczek@prz.edu.pl

At present, there are many different technologies for manufacturing of bioceramic porous materials. The most important of these are: replication of porous matrices, gel-casting of foams, and direct ink writing.

Replication of porous matrices involves mapping the structure of other porous materials into the ceramic material [10]. Replication of porous organic matrices is most commonly used technique in manufacturing of porous ceramic materials. The ceramic mass is deposited on a prepared matrix usually made of a polyurethane sponge. After drying, the polymer substrate is fired and the deposited ceramic mass takes the structure of the substrate. This method can be used to produce materials with high porosity even above 95% and large pore sizes up to several millimetres. The advantage of the replication method is that the pore size can be controlled by using appropriate types of organic matrices. The disadvantage, however, is the voids in the ceramic skeleton resulting from the firing of the polymer substrate which reduces the mechanical strength of the porous ceramics.

Gel-casting of foams makes it possible to manufacture porous ceramics with a high porosity of 60 to 95%, predominantly open [11-12]. These ceramics are characterized by the cellular structure. Spherical-shaped foam cells with diameters of several hundred micrometers are connected to each other by passage pores called "windows" with diameters ranging from a several to a hundred tens of micrometers. The skeleton holding the walls of the foam cells is made of a dense ceramic phase. Ceramic foams produced by the most common method to date, which is the replication of a porous organic matrix do not exhibit such a structure. One of the problems of the replica template method is that the ceramic struts are hollow, due to volatilization of the organic filament used as a substrate. Therefore, the compression and flexural strengths of the ceramics obtained by the gel-casting of foams method are higher compared to ceramics produced by the porous matrix replication technique. The idea of the gel-casting of method is the preparation of ceramic suspension, that contains its basic components such as: ceramic powder, water and dispersant. Next, foaming agent and gelling agent are added to ceramic suspension. Foaming agents reduce the surface tension at the liquid/gas interface, allowing foam to form during the mechanical dispersion of gas in the suspension. Gelling agents, on the other hand, fix the foam structure by creating a strong hydrogel in the liquid phase that separates the gas bubbles. Subsequent technological steps include drying, firing the organic binder and sintering.

Direct ink writing (DIW) belongs to additive manufacturing (AM) methods. The advantage of this technique is the ability to reproduce a pre-designed digital model using a digitally controlled machine a 3D printer, forming it by building layer by layer [13-14]. This makes it possible to obtain complex dense or porous structures that maximally fulfil the requirements of the designers. In addition, the direct ink writing method is one of the best ways to produce individualized implants for bone reconstruction and regeneration, as it allows bone implants to be made for patients based on tomographic or magnetic resonance image. This makes it possible to almost perfectly tailor a shape for an individual patient. The essence of direct ink writing of ceramic materials boils down to designing a computer model of the shape, preparing the ceramic mass with organic additives that modify its rheological parameters, and making the shape using a 3D printer. The next steps are drying, firing the organic additives and sintering.

The aim of this study is to manufacture and characterize porous calcium phosphate ceramics with hierarchical pore structure by gel-casting of foams method using agarose as a gelling agent. The gel-casting of foams method was chosen because according to Jones and Hench [15], the morphology of bioceramics produced by this technique resembles that of natural cancellous bone. Agarose was chosen as the gelling agent because this environmentally friendly biopolymer forms strong physical hydrogels as a result of conformational transitions at about 37°C during cooling the system. In this way agarose forms sufficiently strong gel which is able to withstand the foam weight.

Materials and methods

$Ca_3(PO_4)_2$ powder made by Merck was used in the study. The specific surface area of the powder was equal to 12.8 m^2/g, while the average grain size was 0.81 μm. Sodium polyacrylate, with the trade name Darvan 811 (R.T. Vanderbilt Company Inc. USA) was used as a dispersant at an amount of 0.65% by weight of active ingredient per weight of ceramic powder. Agarose (MEEO Ultra-Qualitaet, Roth) was used as a gelling agent. Surfactants with the trade name Tergitol TMN-10 (Fluka) and Simulsol SL-26 (Seppic) were used for foaming the suspension.

Aqueous suspensions of $Ca_3(PO_4)_2$ at 40 vol% were prepared in a planetary ball mill. After the whole portion of the ceramic powder was introduced into the solution of water with dispersant, mixing was continued for a period of 1h at a speed of 250 RPM. Aqueous solutions of agarose were prepared by dissolving it at 95°C. Aqueous ceramic suspensions containing agarose were prepared by heating the $Ca_3(PO_4)_2$ suspension to 60°C, then the agarose solution of the same temperature was added and next the suspension was thoroughly stirred in a sealed vessel to prevent evaporation of water. The amount of agarose solution added was chosen so that the final concentration of biopolymer in the suspension was 1.5 wt.% per liquid phase. Next, surfactants were introduced in the amount of a few drops (Tergitol TMN-10 and Simulsol SL-26), and then the suspension was foamed with a high-speed mixer in a vessel placed in a water bath at 60°C. The foamed suspension containing agarose was then

poured into a plastic mould and the mould was cooled down using flowing cold water (15°C) to gel the wet foam. The green bodies were then de-moulded and left in room conditions to dry. Additional drying was performed at 80°C. After the drying process, smaller samples of about 12x12x25 mm3 were cut from the shaped β-TCP ceramics and next they were sintered at 1100°C for 2h. After the sintering process the physical and mechanical proprieties of β-TCP were determined as well as the microstructure of porous ceramics was evaluated.

The microstructure of the porous β-TCP ceramics was determined by scanning electron microscopy (SEM) using a Jeol JSM-550 LV microscope based on observations of randomly selected fractured porous samples. Pictures for monitoring the cellular structure were taken for estimation of cell and window sizes. The size distribution of foam cells and windows in the foam cell walls was determined by image analysis from a random selection of ten areas occupied by at least of 50 cells. The size distribution of micropores in the ceramic framework was also determined by image analysis.

The apparent density and the open porosity of the samples were determined by hydrostatic weighting. The average of five samples was taken as the value of open porosity and apparent density. The real density of $Ca_3(PO_4)_2$ powdered foams was determined in a helium pycnometer (Accu Pyc 1330). The total porosity was calculated based on the determined values of real and apparent densities. The compressive strength of the calcium phosphate ceramic samples was determined using an Instron 3345 testing machine. The crosshead speed was 0.5 mm/min. The average of five specimens was taken as the compression strength value of β-TCP ceramics.

Results and discussion

Table 1 summarizes the values of real density of the ceramic skeleton and apparent density, open porosity and total porosity of the β-TCP ceramic foams. The porous samples had high open porosity (86%), and the difference between total and open porosity was less than 1%. High open porosity, as well as adequate pore sizes, are required for implants intended for bone tissue reconstruction.

Table 1.   Real and apparent density, open and total porosity of the β-TCP ceramic foam.

Real density of the skeleton g/cm³	Bulk density g/cm³	Apparent density % of real density	Open porosity vol. %	Total porosity vol. %
3,04 ± 0,01	0,39 ± 0,02	12,9 ± 0,1	86,2 ± 0,4	87,1 ± 0,4

The microstructure of the sintered β-TCP ceramic foam is presented in Fig. 1. It is characterized by a hierarchical pore structure, e.g. the presence of spherical macropores (foam cells) interconnected by passage pores (so-called "windows") located in the common walls of the foam cells (Fig. 1a), as well as micropores in the ceramic skeleton (Fig. 1b).
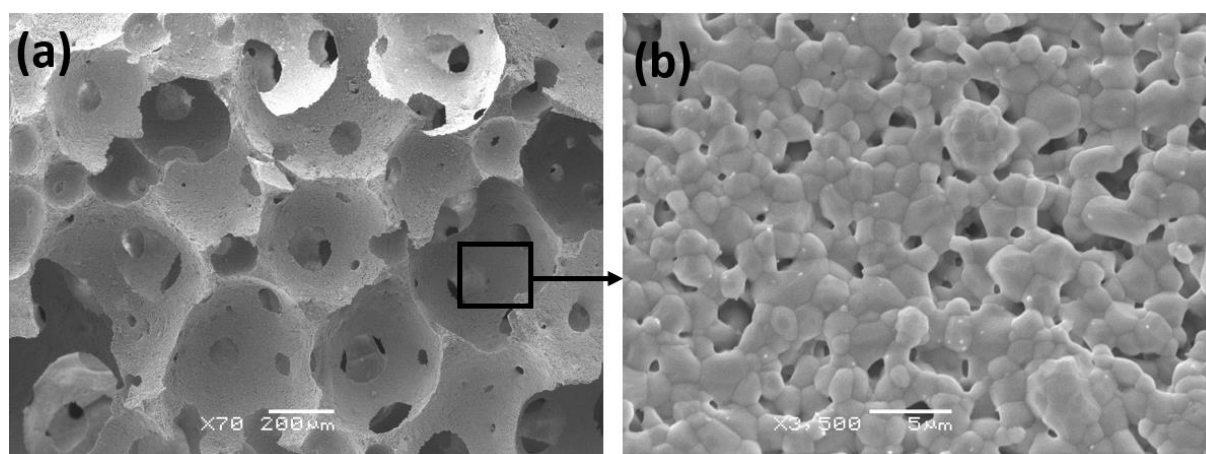

Fig. 1. Microstructure of the calcium – phosphate foams: (a) macropores and connectons between macropores, (b) micropores in the ceramic skeleton.

The image analysis of SEM micrographs showed that the foams had the cell size from 250 to 800 µm with the dominant population in the range of 400 - 450 µm (Fig. 2). On the other hand, the connections between macropores (so-called "windows") had the diameters in the range from 30 to 355 µm with the dominant population in the range of 105 - 155 µm (Fig. 3). According to the literature, optimal ingrowth of bone tissue is demonstrated by scaffolds

having macropores size in the range of 100 - 500 μm [5]. Another important factor is the dimension of window diameter between connected cells, as too small they can prevent osteoconduction by the scaffold structure. The optimal window size should be above 50 μm [5]. In addition to the macropores (cells and cell windows), micropores occurring in the ceramic skeleton also improve bone growth into the scaffold. It is now accepted that micropores located in the ceramic skeleton provide sites for protein adsorption and accelerate the release of degradation products, which facilitates interactions between the ceramic skeleton and bone cells. Moreover, capillary forces generated by the micropores can improve the adhesion of bone-bound cells on the surface [16-18]. The micropores obtained in this work had equivalent diameter ranging from less than 1 to about 3 μm (Figure 1b).
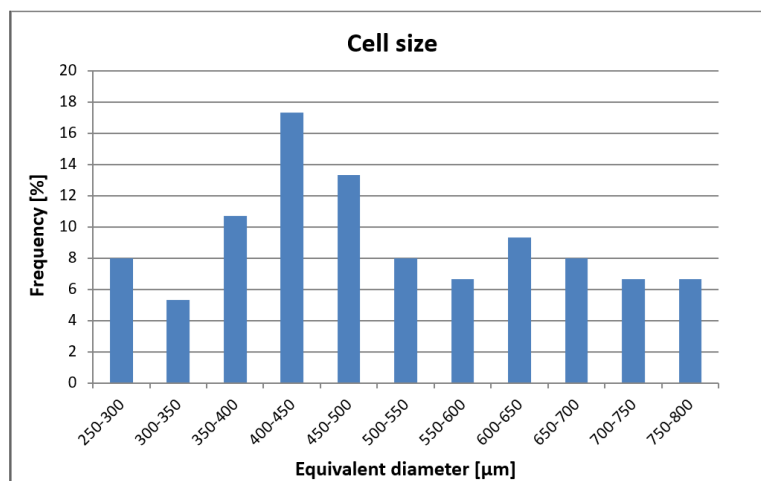

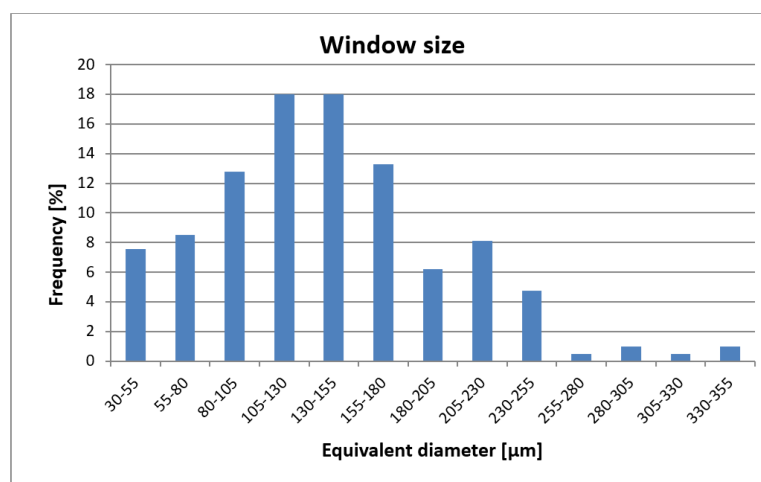Fig. 2. Cell size distribution for the calcium phosphate foam having total porosity of 87%.

Fig. 3. Window size distribution for the calcium phosphate foam having total porosity of 87%.

Porous β-TCP is characterised by low mechanical strength and therefore it is unsuitable for load-bearing part of bone implants [19]. Fig. 4 shows a representative stress–strain plot for the β-TCP foams. Compressive strength tests of calcium phosphate foams carried out in this work showed that these materials, like other ceramic cellular materials, are characterized by the occurrence of three zones on the compressive stress-strain plot. At low stress, the foam deformed in an elastic manner (Fig. 4, zone A). Increasing the stress to a certain value caused gradual cracking of the cells but not in a catastrophic manner, as in the case of dense ceramic materials. When the stress reached a critical value, the sample started to crack and the stress fell onto the plateau region. A wavy oscillations pattern can be observed in the plateau region that can be attributed to the layer by layer fracture of the cell walls (Fig.4, zone B). Then, there was an area of compaction causing the destruction of the foam due to brittle fracture (Figure 4, zone C). The obtained in this work compressive strength value of calcium phosphate foam of 0.5±0.1 MPa is typical for highly porous β-TCP ceramics [20].
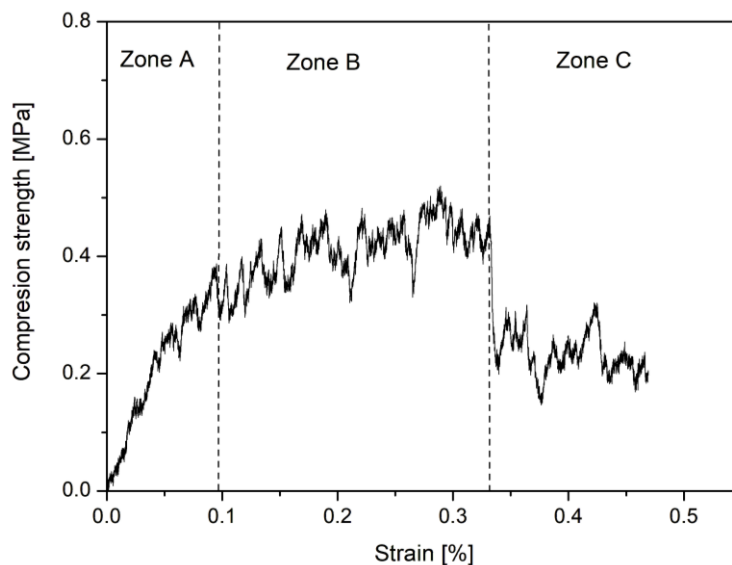
Fig. 4. Compression stress – strain curve of the calcium – phosphate foam having total porosity of 87%.

Before being used as implants, the manufactured porous calcium - phosphate ceramics require *in vitro* and *in vivo* biological evaluation.

Summary and conclusions

In this study, foamed $Ca_3(PO_4)_2$ ceramics with the total porosity of 87% were produced by gel-casting of foam method using agarose as a gelling agent. The material was characterized by a hierarchical pore structure, i.e. the presence of spherical macropores, connections between macropores (so-called "windows") and micropores in the ceramic skeleton.

Diameters of the cells were contained in the range from 250 to 800 μm with the dominant population in the range of 400 - 450 μm.

Diameters of the connections between macropores (so-called windows) were contained in the range of 30 to 355 μm with the dominant population in the range of 105 - 155 μm.

The micropores found in the ceramic framework were characterized by equivalent diameters ranging from less than 1 to about 3 μm.

The compressive strength of the calcium phosphate foam was 0.5 MPa.

Acknowledgement

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