

## A METHOD OF FABRICATION OF POROUS CARBONATED HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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### **Abstract**

*A method to produce porous carbonated hydroxyapatite ceramics was developed which is based on vacuum impregnation of cellular polyurethane (PU) matrixes with a ceramic slip. The polyurethane foams were burnt off and the samples were converted into porous carbonated hydroxyapatite (CHA) ceramics by sintering in a furnace at 600 to 650°C using a sintering additive. The ceramics had 60–90% interconnected porosity, necessary to facilitate cell seeding and fixation which is an important requirement for use in bone tissue engineering.*

*The optimal composition of ceramic slip and the sintering conditions were found. PU foams with a different number of pores per inch (ppi) were used and the strength testing of ceramics was carried out. It is suggested that the experimental ceramics would be useful in bone replacement and reconstruction.*

**Keywords:** *ceramics, scaffold, carbonated hydroxyapatite, resorption*

### **INTRODUCTION**

Porous calcium phosphate ceramics are expected to play an important role in treating bone problems with the emerging tissue engineering approach, as a template for cell interactions and the formation of bone-extracellular matrix to provide structural support for achieving bone tissue regeneration [1]. Among the different biomaterials considered as scaffolds for bone tissue engineering, calcium phosphate based materials have proved to be of great interest - given their osteoconductivity and ability to integrate with the bone tissue [2-6]. The high porosity of ceramic matrix and the high roughness of the surface allows migration and proliferation and fixation of osteoblasts and mesenchymal cells for bone tissue formation. In addition, a porous surface improves mechanical interlocking between the implant biomaterial and the surrounding natural bone, providing greater mechanical stability at this critical interface [7].

In the course of fabrication of such ceramics it is necessary to take into account the important properties that material must possess. The minimum pore size required to regenerate mineralized bone is generally considered to be greater than 100  $\mu\text{m}$  [8]. Large pores (100-150 and 150-200  $\mu\text{m}$ ) showed substantial bone ingrowths. Smaller pores (75-100  $\mu\text{m}$ ) resulted in ingrowths of unmineralized osteoid tissue. Smaller pores (10-44 and 44-75  $\mu\text{m}$ ) were penetrated only by fibrous tissue [8]. Porosity can, however, adversely affect important mechanical characteristics of a ceramic.

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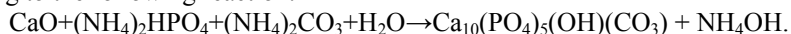
On the other hand the biological apatites are always calcium deficient and carbonated, being then carbonate hydroxyapatites. Also, the apatite crystals which form the natural bones are smaller than 500 Å in size [9]. The presence of  $(\text{CO}_3)^{2-}$  in their structure is the main source of lattice distortion, creating microstresses and crystalline defects in its vicinity, which play a fundamental role in the solubility, particularly rises of osteoconductivity. So we find it more promising to use ceramics based on carbonated hydroxyapatite to produce porous ceramic with high resorption properties.

The work was aimed at the development of a method to fabricate porous carbonated hydroxyapatite ceramic scaffolds for bone tissue engineering. The microstructures, porosity, mechanical properties of resulting materials were investigated with respect to varying fabrication conditions. The ceramics can find application in bone replacement and reconstruction.

## EXPERIMENTAL PROCEDURE

### Powder synthesis

Carbonated hydroxyapatite (CHA) was synthesized by solid-state reaction using calcium oxide, ammonium hydrophosphate and ammonium carbonate as starting reagents according to the following reaction:



Details of the preparation route are described elsewhere [10]. The resulting precipitate was dried at 100-120°C and calcined at 600°C. The powder was mixed with 3 wt.% of a sintering additive based on  $\text{K}_2\text{CO}_3$ . The specific surface area of CHA powders was 30-40 m<sup>2</sup>/g.

### Process for producing porous scaffolds

CHA powder was added to polyacrylamide and gelatin 10% wt. water solutions to prepare slips of 1 g of powder per ml.

Polyurethane (PU) reticulated foams were used as porous matrices, which were completely burnt out during sintering. To produce ceramic samples three kinds of PU foams were used with different pore sizes, mm: 0.05-0.15 (a); 0.1-0.5 (b); 1.0-2.0 (c) to form the PU cylinders of 15 mm diameter and 30 mm height. Also small PU foams of 4 mm × 4 mm × 4 mm with pores only of 0.1-0.5 mm (b) were used to prepare the ceramic granules.

The PU matrices were substantially impregnated with ceramic slips based on PAA and gelatin by a vacuum impregnation method. The ceramic suspension settled on the matrix surface and repeated the matrix structure. The samples were dried at 60-80°C during 24 h and were sintered at 800-850°C. The sintered samples had porosity 60-90 vol.% depending on the initial PU foams. The photos of final materials are presented in Fig.1.

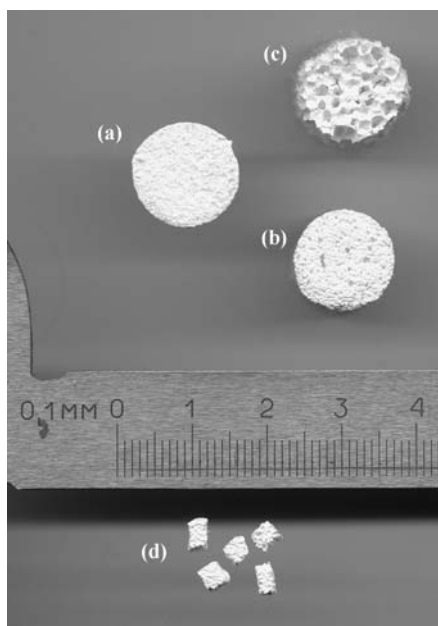


Fig.1. Porous CHA ceramic samples made from the foams with different pore size in mm: (a) – 0.05-0.15; (b,d) – 0.1-0.5, (c) – 1.0-2.0.

## Methods

The specific surface of the powders was determined by the Brunauer–Emmett–Teller (BET) method using a Tristar surface area analyzer, Micromeritics Ltd. The method is based on the measurements of the quantity of nitrogen gas adsorbed onto or desorbed from a solid surface at equilibrium vapor pressure. The phase composition of the samples was determined using  $\text{CuK}_\alpha$  radiation by X-ray diffraction (XRD), Shimadzu XRD-6000 diffractometer. The microstructures of the sintered HA ceramics were studied by scanning electron LEO 1420 microscope. The total porosity of ceramics was determined on the basis of density, geometric parameters and weight of material in the same way as described in [11]. The strength tests were carried out with an Instron 5581 stiff testing machine.

## The strength test

The test was performed to investigate the compressive properties of different types of ceramic porous materials as cylinder-shaped samples and granules.

The strength experiments for cylinder-shaped ceramic samples were carried out by the conventional way of compressive strength measurement. The testing for granules was carried out in the same way as in [12]. The testing device is shown schematically in Fig.2. It consists of a plunger cylinder of 15 mm diameter and 100mm length which is pushed into a hollow cylindrical container of equivalent internal diameter. The bottom of the cylinder is closed with a 15 mm diameter disc which can be easily removed after the compression experiment to retrieve the granules. The granules were manually inserted into the die and pushed down slightly by the weight of the steel plunger cylinder. The cross-head speed of the loading device was 2 mm/min. For each test the load cell was 2000 N.

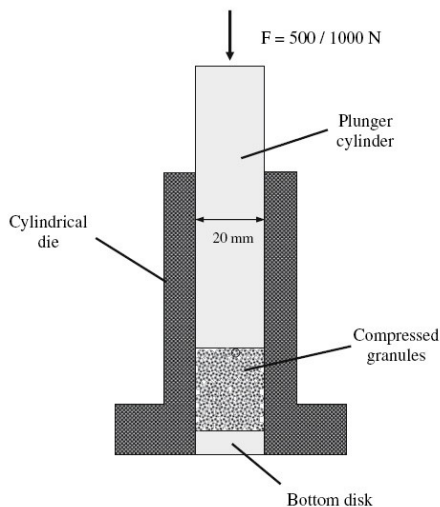


Fig.2. Schematic representation of compressive test.

## RESULTS AND DISCUSSION

The characteristics of ceramic cylinder samples prepared from PAA slip were compared. Figure 3 shows the compressive strength of ceramic cylinders made from PU foams with different pore sizes. The results are presented in Table 1 and satisfy the principle that with increasing of porosity the strength value decreases. Thus, the ceramics made from the foams (b) possess optimal characteristics: compressive strength up to 3 MPa and total porosity 50-60%. The ceramics made from foams with the smallest pores (a) had lower strengths, down to 1 MPa and high porosity of 60-70% as a result of the large amount of closed pores of the initial foam that were impervious to ceramic slip during impregnation. The ceramics made from the foams with the largest pores (c) had long thin and fragile necks, adversely affecting mechanical properties (1 MPa) and high porosity (80-90%). The cylinder-shaped ceramic made from the gelatin suspension possessed low mechanical properties, such that the samples were destroyed after sintering.

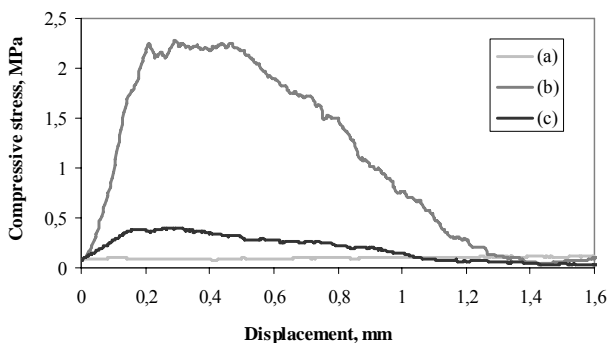


Fig.3. Force-deformation diagram for strength testing of CHA ceramic cylinder samples made from foams of different pore dimensions in mm: (a) - 0.05-0.15; (b,d) - 0.1-0.5, (c) - 1.0-2.0.

Tab.1. Basic characteristics of porous ceramics.

Sample No.	Pore dimension of foam [mm]	Compressive strength [MPa]	Summary porosity [%]
(a)	0.05-0.15	0.5	60-70
(b)	0.1-0.5	2.3	50-60
(c)	1-2	< 0,5	80-90

Thus, the PU foams with pores of 0.1-0.5 mm (b) were used further to prepare ceramic granules. The strength results for ceramic granules are presented in Fig.4. The granules made from polyacrylamide (PAA) suspension had the lower compressive strength of 5.5 MPa up to displacement of 10 mm compared to 10 MPa for the granules made from the gelatin suspension. This indicates that gelatin solution is more suitable to fabricate ceramic porous granules by impregnation of PU foams in comparison with PAA solution. Figures 5 and 6 demonstrate the difference between the microstructural features of the ceramics and granules. During the impregnation of cylindrical PU foams, the ceramic slip coated and spread and streamed down on the neck surfaces, creating a ceramic replica of the reticulated foam material. When the granules were fabricated, the ceramic slurry filled the pore space and, after sintering, the resulting material preserved the channels of pores.

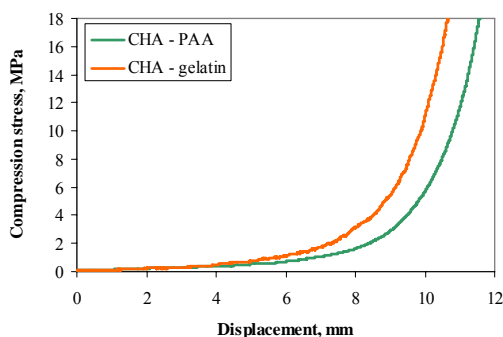


Fig.4. Force-deformation diagram for strength testing of ceramic granules.

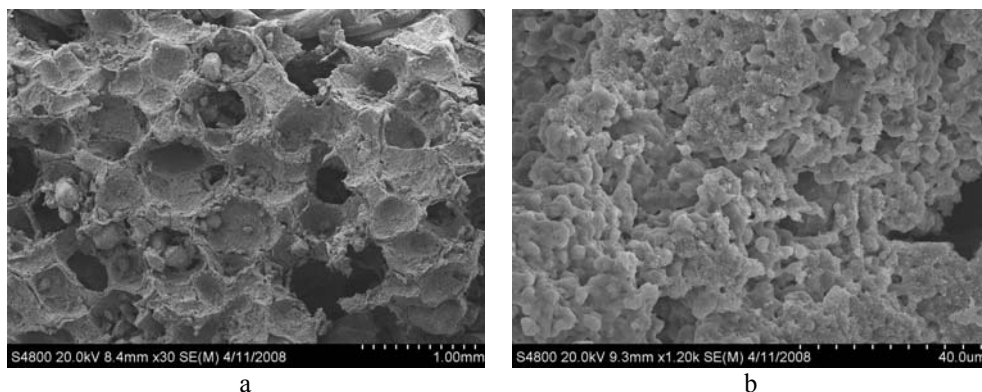


Fig.5. SEM micrographs of ceramic cylindrical samples.

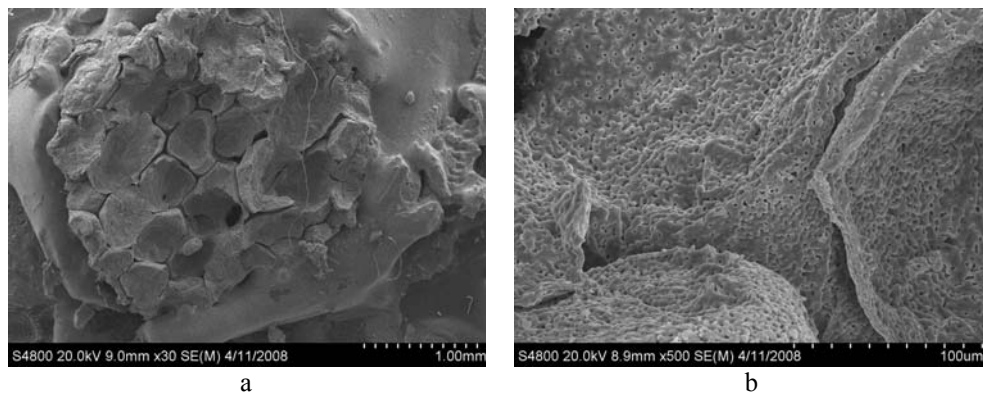


Fig.6. SEM micrographs of ceramic granules.

In this study the experiments for cylindrical samples and granules were carried out in a parallel way. It should be noted that the strength of the cylindrical samples made from PAA-based suspension is higher than of the samples fabricated from gelatin suspension. In contrast, granules made from gelatin-based ceramic slurry demonstrated better properties than the granules made from the PAA-solution. The difference can be explained by the behavior of polymer suspension during drying. The difference between gelatin and polyacrylamide reveals a shrinking effect. While water evaporated from the suspension, the polymer substrate began to gather the powder particles onto the foam surface. Experimentally the tie force of gelatin suspension is stronger than for the polyacrylamide suspension. The cylindrical dried samples made from gelatin suspension were more fragile and cracked more than the samples made from PAA-suspension. In contrast the green granules made from the gelatin suspension were stronger, due to the possibility of deformation of the small-size foam.

## CONCLUSIONS

The method of impregnation of porous matrix with ceramic slip can be used to make porous carbonated hydroxyapatite samples with interconnected porosity in amounts up to 90%. Carbonated hydroxyapatite ceramics of various shapes, i.e. cylinders of 12 mm×24 mm and granules of 3 mm × 3 mm × 3 mm with compressive strength up to 3 MPa and 18 MPa can be prepared at reduced sintering temperatures down to 800-850°C by just using sintering additives. It can be concluded that the experimental ceramics could be useful as bone defect fillers in bone tissue engineering.

## Acknowledgments

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