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DEVELOPMENT OF POROUS CALCIUM PHOSPHATE BONE SCAFFOLDS FOR DRUG DELIVERY

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Resume: Synthetic calcium phosphates (CaP) are widely used as bone scaffolds due to their biocompatibility and osteoconductive properties. To be used as drug delivery systems, the pore structure of the scaffolds needs to be controlled in terms of porosity, pore size and pore interconnectivity.

In this research hydroxyapatite/β-tricalcium phosphate biphasic mixture was synthesized by wet precipitation reaction. Porous bioceramics was obtained using highly viscous/plastic mass foaming, where polyol was used as a liquid phase for the mass preparation and H_4NHCO_3 was used as a pore forming agent. Porous ceramics obtained had the total porosity in range from 30 to 70 %. Gentamicin in porous bioceramic scaffolds was incorporated using vacuum infiltration. Results obtained demonstrated pore sizes in range from 60μm to 300μm in diameter, interconnectivity of pores, relatively dense pore walls and drug release within more than 6 hours.

Key words: bioceramics; bone scaffolds; drug delivery systems; calcium phosphate ceramics.

1. INTRODUCTION

The main driving force behind the use of CaP as bone substitute materials throughout the body, covering all areas of the skeleton, is their chemical similarity to the mineral component of mammalian bones and teeth [1, 2]. CaP is known to be osteoconductive and support osteoblast adhesion and proliferation, however, the brittleness and low strength limit the CaP bioceramic wider applications in hard tissue implants [3, 4].

The development of biphasic calcium phosphate (BCP) bioactive ceramics for bone implants involves control of the process of biomaterial resorption and structure. The concept of this kind of bioceramics is based on an optimum balance between more stable phase – hydroxyapatite (HAp) and more soluble phase – β-tricalcium phosphate (TCP) [5, 6]. Various synthesis routes, including wet chemical synthesis (such as precipitation, hydrothermal, hydrolysis and sol-gel techniques) have been developed for the preparation of calcium phosphate powders, which have to present desirable characteristics, such as, crystallinity, stoichiometry, morphology, surface area particle size for specific applications [7, 8]. All these properties are dependent on synthesis method and its conditions. Physico-chemical characteristics of synthesized CaP have significant effect on the final material quality (phase composition, density, thermal stability, microstructure, mechanical properties, dissolution behaviour and cellular response in living body) after thermal treatment.

Mostly the CaP porous forms have been used as bone grafts to mimic the porous architecture of bone and to provide appropriate space for bone ingrowths, promoted by interconnectivity of pores [9, 10]. Up to know several methods for porous CaP bioceramics production have been developed: pyrolysis of organic particles, foam sintering, gel-casting, polymeric sponge technique, coextrusion process, leaching, hydrothermal exchange, for instance using corals, marine invertebrates, bicontinuous microemulsion technique, rapid prototyping techniques, freeze-gel casing, foam-gel technique, sacrificial template method, direct foaming methods [11 - 13]. Selection of the processing route for the production of porous ceramics depends primarily on the final properties and application. To obtain porous structure, not only synthetic CaP, but also biogenic materials like bovine bone, corals and even wood are used for bone graft production [14].

Bone replacement surgery usually is followed by high systemic doses of antibiotic drug substances for prolonged periods of time [15, 16]. This treatment is poorly selective, so that damage can occurs to the healthy tissues and organs, different from the intended target [17, 18]. In addition, high drug doses is required to achieve the desired effect, thus implantable delivery tools, able to release the active substance in a controlled way and local area are of clinical importance

In the current research an approach to the fabrication of porous bone graft, exhibiting bone regeneration function as well as the local drug delivery was made.

2. THE BODY OF THE ARTICLE

Materials and methods

CaP powder used for bone graft preparation was prepared by a precipitation reaction between calcium hydroxide suspension (CaO, Riedel-de Haën®, Germany) and orthophosphoric acid solution (H_3PO_4, 85%, Sigma-Aldrich, Germany). Calcium oxide was suspended in distilled water and milled at rotation speed of 300 rpm with a Pulversette 5 planetary mill (Fritsch, Idar-Oberstein Germany) to obtain homogenous calcium hydroxide suspension. The precipitation reaction took place in a 2 l reactor, equipped with stirrer, electrical heater with thermostat, combined pH electrode and Titronic® system for acid solution addition. Acid solution was added to the calcium hydroxide suspension with slow addition rate ~0.75 ml/min under vigorous stirring. CaP were synthesized using appropriate synthesis conditions – pH of synthesis medium (pH 7,7) and temperature (45°C) The suspension was aged for 20 hours at room temperature. After aging the suspension was filtrated in a Buchner funnel and dried at 105°C.

The dried precipitate was milled to obtain a fine powder. To attain a highly viscous/plastic mixture, powder was mixed with liquid phase - polyol. Different amounts of H_4NHCO_3 were added to the plastic mass as pore forming agent. Prepared mass was placed in cylindrical moulds and then heated increasing temperature from 40 to 110 °C for proceeding the foaming process and particular release of organic additive from the sample. Samples were sintered at 1150 °C for two hours. Ceramic samples were investigated using X-ray diffractometry (XRD) and scanning electron microscopy (SEM).

Gentamicin sulphate (Sigma-Aldrich) was used as a model drug for drug delivery system (DDS) preparation. Vacuum impregnation was applied for drug incorporation in porous bioceramics. Release of gentamicin was determined via thin layer chromatography method.

Results and discussions

The effect of synthesis temperature and final pH on the end product was examined by XRD. HAp/β-TCP ratio in BCP ceramics was calculated using semi-quantitative XRD analysis according to the intensities of the most intense diffraction peaks of HAp and TCP at 2θ=31.71° and 31.03° respectively (see Fig. 1 a). SEM was used to evaluate the microstructure of BCP bioceramics sintered at 1100°C for 1h (see Fig. 1 b).

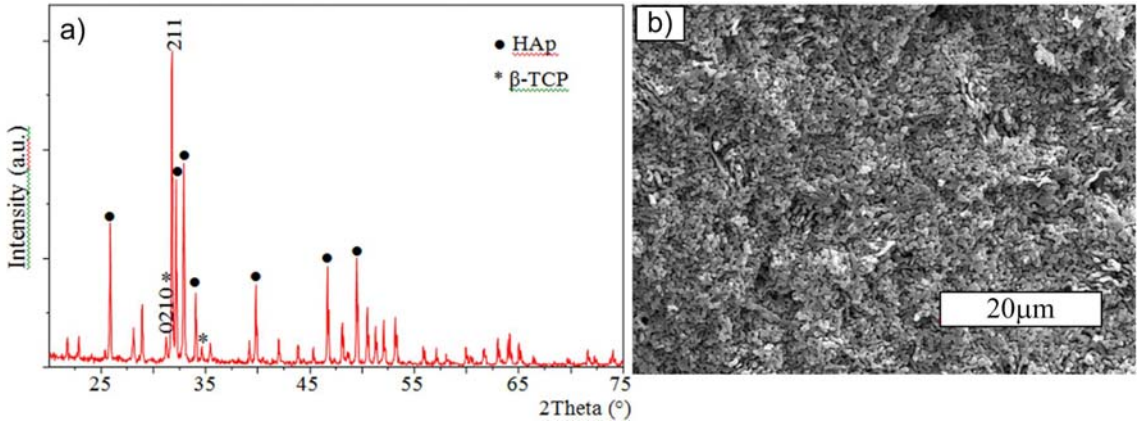

Figure 1. BCP bioceramics (HAp/β-TCP : 90/10): a) XRD patterns; b) SEM micrographs

The results showed that obtained BCP ceramic comprise of HAp/β-TCP mixture in ratio 90/10 and forms homogenous fine-grained microstructure.

For the production of porous bone grafts, dry powder after precipitation reaction was used. It was established that by varying amount of pore forming agent in the plastic mass, it is possible to achieve different porosities after sintering. The total and open porosity of porous bioceramics obtained was determined using Archimedes method. As seen in Fig. 2.a), the total porosity of bioceramics ranges from 28 to 70 %, while the open porosity is in range from 25 to 50%. Increasing amount of pore forming agent, it was observed that the total porosity increased more rapid than the open one. As it is seen in SEM microphotograph (see Fig. 2.b) porous bioceramic pore sizes are in range from 60μm to 300μm in diameter, pores are interconnected and pore walls are relatively dense. Pore sizes and interconnectivity are sufficient for the porous bioceramics to be used as bone grafts.

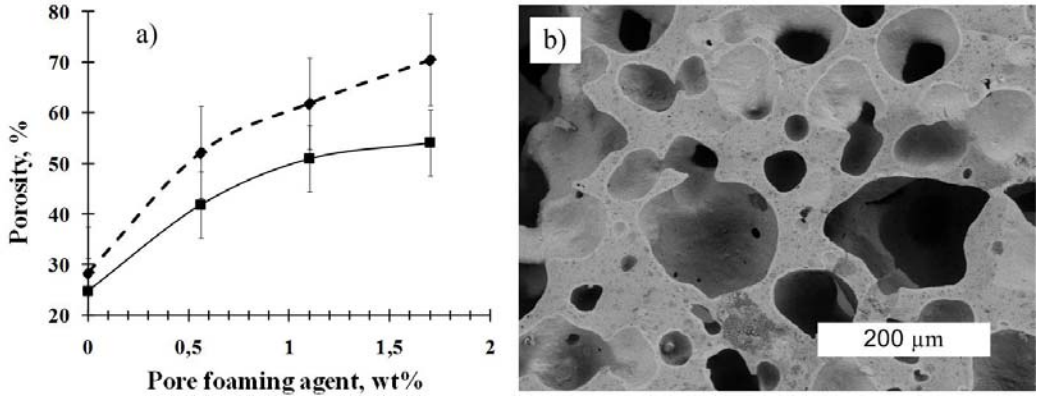

Figure 2. Porous bioceramics: a) porosity dependence on amount of pore forming agent after sintering (total porosity – dashed line, open porosity – solid line); b) SEM microphotograph of fracture surface

To prepare local sustained release drug delivery system, the porous bioceramic scaffolds were impregnated with gentamicin water solution. Three impregnation cycles were applied to obtain drug/BCP composite containing 9mg of gentamicin. *In vitro d*rug release profile was studied for 48h in simulated body fluid at 37^{o}C ± 0.5^{o}C. Gentamicin was gradually released from bioceramic scaffolds and after 1h of dissolution, 40% of active substance was already transferred into the dissolution media. During the next five hours 70% of drug was released, but the maximum amount of gentamicin released in dissolution media was reached within 30min (see Fig. 3).

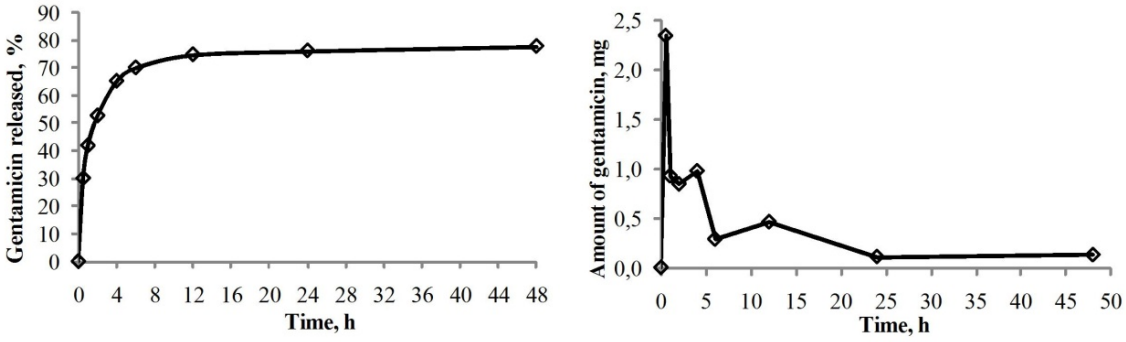

Figure 3. Gentamicin release from CaP scaffolds

3. CONCLUSION

The use of wet precipitation method for synthesis of BCP at reported conditions leads to the formation of HAp/β-TCP mixture in ratio of 90/10.

Highly viscous/plastic mass foaming is a perspective method for the preparation of porous CaP ceramics. Obtained ceramics met the preconditions for the preparation of scaffolds for bone regeneration and local drug delivery.

Gentamicin was gradually released from the bioceramic scaffolds and the drug release was sustainedfor more than 6 hours 70% of drug was released.

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რეზიუმე: სინთეტიკური კალციუმის ფოსფატები (CaP) ფართოდ გამოიყენება ძვალთან ბიოშეთავსებადობის და ოსტეოგამტარობის გამო. წამლის შესაყვან სისტემად გამოყენებისას ხდება ჩონჩხის ფოროვანი სისტემის კონტროლირება ფორიანობის, ფორების ზომების და ურთიერთკავშირის მიხედვით.

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