EFFECT OF CALCINATION PARAMETERS ON BEHAVIOR OF BONE HYDROXYAPATITE IN ARTIFICIAL SALIVA AND ITS BIOSAFETY

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Abstract— Objective: Being the main inorganic constitution of hard tissues (bone and teeth), calcium phosphates have been attractive in medical and dental applications in hard tissue repair. The evaluation of predicted osteointegration of implant material with bone most often begins with in vitro tests simulating living organism environment. The paper presents the behaviour in artificial saliva of hydroxyapatite (HAp) obtained from pork bone sludge. Methods: Evaluation of physicochemical properties of HAp (raw powders and sinters obtained from meat industry) after immersion in artificial saliva by various characterization techniques (eg. X-ray diffraction (XRD), FTIR, SEM and EDS methods) indicates their potential application in medicine and dentistry. Conclusions: On the basis of bioactivity tests in in vitro conditions, it was found that hydroxyapatite of natural origin exhibits the composition stability during incubation in artificial saliva.

Keyword— Hydroxyapatite Natural Origin, Biomaterial, Calcination Process, Artificial Saliva, Incubation.

I. INTRODUCTION

Hydroxyapatite bioceramic (HAp) has the highest biocompatibility of all synthetic inorganic implant materials. Because of the similarity to chemical composition of natural apatite in bone, hydroxyapatite does not irritate the surrounding tissue, does not cause acute or chronic inflammation, instead it stimulates bone repair processes, which enable the creation of chemical bond at the implant-bone interface [1, 2].
Discussing hydroxyapatite it is important to consider its behavior in the living body environment. Typical reaction of living organism to a foreign body is its destruction by resorption. Resorption in a living organism is a complex process and involves chemical and biological disintegration stimulated by osteoclasts adsorbed on both bone and implant materials. In the resorption process, an important role plays macrophages capable to absorb disintegrated particles of implant materials. Simultaneously, due to the activity of osteoblasts, new bone has been formed [3-5].
Biodegradation of some implanted materials is associated with dissolution of intergranular necks, separation of single particles from bulk materials and their absorption by phagocytes [4, 6]. The above processes (especially biodegradation) have adverse effects such as mechanical weakness of implant or even its complete destruction [1, 5]. The rate of these processes depends on phase and chemical composition, porosity and heat treatment conditions [1, 3]. HAp exhibits high resistance to resorption and biodegradation tests in contrast to another calcium phosphate - tricalcium phosphate (TCP) with much higher tendency to degrade in the human body [6].
The tendency of apatitic calcium phosphate to resorption decreases with increasing Ca/P molar ratio. The greatest degree of resorption is observed for a hydroxyapatite with Ca/P molar ratio in the range of 1.3-1.4, while at a ratio in the range of 1.8-2.0 this phenomenon is almost unnoticeable [7, 8]. Resorption process also favors the presence of the amorphous phase in the intergranular spaces, the presence of secondary phases and a low degree of crystallinity [5]. Moreover, porous materials are more prone to resorption and biodegradation processes compared to dense materials [9]. Also, heat treatment of hydroxyapatite materials has significant impact on their stability in body environment - HApbioceramics exposed to higher temperatures exhibit a higher stability in body fluids [10].

II. Experimental part

2.1. Hydroxyapatite preparation

An animal origin hydroxyapatite was applied. Detailed preparation method was presented in previous paper [26]. In brief, a raw bone material was preliminary defatted and deproteinized by hydrolysis in lactic acid. Bone sludge obtained was calcined in two-step process in a chamber oven or in a rotary kiln. Preliminary calcination was carried out at a temperature of 650°C in a chamber oven for 3 hours and in a rotary kiln for 40 minutes. In the second stage of the process material was re-calcined in a chamber oven at temperatures 750, 850 and 950°C in air atmosphere for different times (2.0, 2.5, 3.0 h) or in a rotary kiln at three temperatures: 750, 850 and 950°C.

2.2. Characterization Techniques

The molar ratio of Ca/P was determined by chemical analyses of HAp samples. Calcium was analysed by complexometry titration (EDTA in the presence of

calcein and thymolphthalein as indicators), while phosphorus as a P–Mo–V complex by colorimetry (Marcel Media UV-Vis spectrophotometer). The phase composition of the samples was analysed with the use of X-ray diffraction with Philips X'Pert diffractometer equipped with a graphite monochromator PW 1752/00, Cu Kα 1.54 nm, Ni filter (40 kV, 30 mA).

2.3. Bioactivity in vitro tests

For assessment of bioactivity in in vitro conditions 1.1 g of calcined hydroxyapatite powders were formed in cylindrical disks with 13 mm diameter by the uniaxial compaction in a steel mould at a pressure of 74 MPa using hydraulic press. The compacts were sintered at 950°C in air for 3 h.

The assessment of in vitro bioactivity was carried out in artificial saliva prepared according to [27]. The immersion study was carried out at 37°C by soaking the discs placed vertically in a plastic container with 40 mL of fluid. The ion concentration of artificial saliva (mmol) is presented in table 1. The time of soaking was 62 days.

To evaluate the behavior of hydroxyapatite in stimulated oral environment, pH measurement around hydroxyapatite sinters were performed at 25°C every day for the first week of immersion and subsequently once a week. Starting on the 13th day the weight changes of sinters were investigated once a week. After immersion time the discs were removed from the solution, washed with distilled water, dried at 105°C and analyzed.

III. RESULTS AND DISCUSSION

All sinters incubated in artificial saliva showed a weight gain (Fig. 1). The greatest weight gain was obtained in the case of HAp calcined in the rotary kiln, in 750°C - 0.019% after 62 days of incubation. The lowest weight gain was reported for sinters obtained from the chamber oven with temperature and time of calcination 850°C and 2 h, respectively - 0.008%. For samples obtained from HAp calcined in the chamber oven, the weight gain achieved level in the range 0,012-0,015%. The weight gain of samples from rotary kiln tended to loss with increasing calcination temperature.

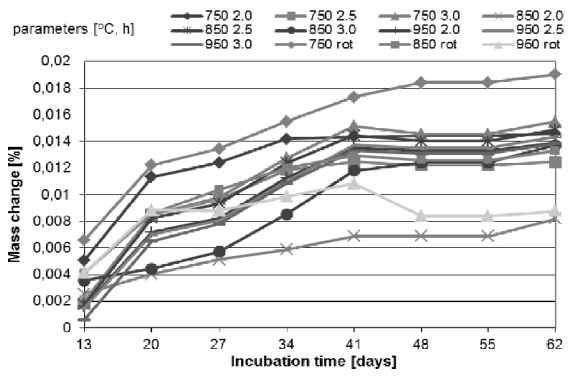


Fig. 1. Weight changes of hydroxyapatite sinters depending on immersion time in artificial saliva

In next step, in all sinters before and after inculabion in artificial saliva, calcium and phosphorus content was analyzed for calculation of Ca/P molar ratio, the factor important in phosphate bioceramics (Fig. 2). In all samples after immersion the results showed a decrease in Ca/P molar ratio to a level of 1.65-1.70. This phenomenon was probably caused by appearance of small amounts of calcium-deficient hydroxyapatite and another calcium phosphate – TCP with lower Ca/P molar ratio [22-23]. The most probable cause of the observed decrease in calcium content may be incorporation of HPO42- ions into crystal lattice formed as a result of reaction between PO43- groups dissolved from HAp and H+ ions from medium.

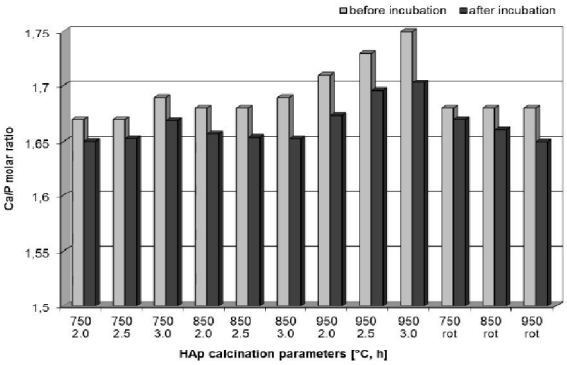


Figure 3. Ca/P molar ratio in HAp sinters before and after incubation in artificial saliva

The phase analysis of HAp samples after 62-day period of immersion in artificial saliva was condacted. XRD patterns (Fig. 3) showed only reflections corresponding to hydroxyapatite. However, the balance of calcium and phosphorus content suggests possibility of secondary phases with lower Ca/P than in HAp molar ratio could be expected, but because of low content, their presence has not been confirmed by X-ray diffraction method.

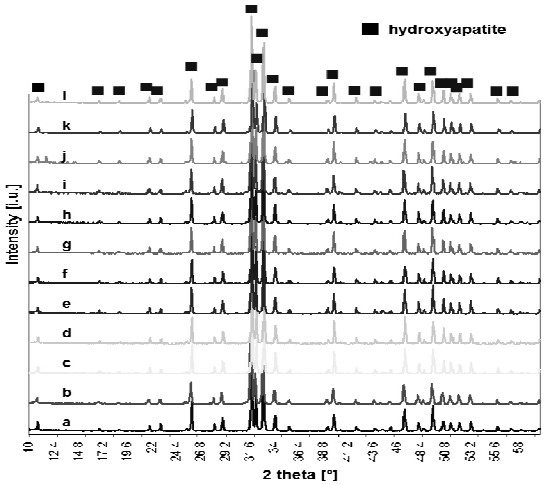


Figure 4.Figure 4. Diffractograms of HAp sinters after 62-day immersion in artificial saliva obtained from HAp calcined in different parameters (°C; h; kiln): (a) 750; 2.0; CO* (b) 750; 2.5; CO (c) 750; 3.0; CO (d) 850; 2.0; CO (e) 850; 2.5; CO (f) 850; 3.0; CO (g) 950; 2.0; CO (h) 950; 2.5; CO (i) 950; 3.0; CO

(j) 750; ROT (k) 850; ROT and (l) 950; ROT, *CO-chamber oven, **ROT- rotary kiln**

CONCLUSIONS

Evaluation of physicochemical properties of HAp of natural origin (raw powders and sinters obtained from meat industry) after immersion in artificial saliva indicates their potential application in medicine and dentistry, however further research, including in vivo studies, are necessary. On the basis of bioactivity tests in in vitro conditions, it was found that hydroxyapatite of natural origin exhibits the composition stability during incubation in body fluids. However, significant influence of calcination parameters on behavior of HAp upon contact with simulating body environment is observed: 1. All immersed sinters exhibit tendency to increase its mass after 62 days of incubation in simulated body fluid. 2. The XRD analysis of HAp confirmed stability of the material - XRD patterns showed only reflections corresponding to hydroxyapatite. Evaluation of calcium and phosphorus content suggests some possibility of phases with lower Ca/P molar ratio than in HAp, however, their content is below the detection limit.

REFERENCES

[1] Knychalska-Karwan Z, Ślósarczyk A. Hydroxyapatite in dentistry, Cracow: Krakmedia; 1994

[2] Ducheyne P, Qiu Q. Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function. Biomaterials 1999;20:2287-03.

[3] Ślósarczyk A. Hydroxyapatite bioceramics, Cracow: Ceramic Bulletin No. 13, Polish Ceramic Society; 1997

[4] Błażewicz S, Stoch L. Biocybernetic and biomedical engineering 2000, Tome 4 – Biomaterials, Warsaw: Academic Publishing House EXIT; 2003

[5] Marciniak J. Biomaterials, Gliwice: Silesian University of Technology Publishing House; 2002

[6] Pollick S, Shors EC, Holmes RE, Kraut RA. Boneformation and implant degradation of corallineporousceramicsplaced in bone and ectopicsites. Int J OralMaxillofacSurg 2005;53:2835-45.

[7] Ślósarczyk A, Pikarczyk J. Ceramic materials on the basis of hydroxyapatite and tricalciumphosphate. CeramInt 1999; 25:561-5.

[8] Henkel KO. A newclass of materials rebuild the bonetissue, unsinteredhydroxyapatitewithout the addition of β-tricalciumphosphate. Dental Materials 2006;3:28-9.

[9] Święcicki Z. Bioceramic for orthopedics, Warsaw: NationalInstitute for TechnologicalResearch PAN, Publishing Cooperative; 1992

[10] Landi E, Tampieri A, Celotti G, Langenati R, Sandri M, Sprio S. Nucleation of biomimeticapatite in synthetic body fluid: dense and porousscaffold development. Biomaterials 2005;26:2835-45.

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