Effect of calcination conditions of pork bone sludge on behaviour of hydroxyapatite in simulated body fluid

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Abstract. The paper presents *in vitro* (in SBF) behaviour of hydroxyapatite (HAp) obtained from pork bone sludge from meat plant via two essentially different calcination methods using a stationary, electrically heated chamber oven and enlarged laboratory scale rotary kiln designed by the authors, heated by gas combustion methods enabling application of different set of physical parameters such as temperature, holding time-differing according to type of the kiln. HAp ceramic discs, after 62-days of incubation in SBF at 37 °C, characterized by X-ray diffraction (XRD), FTIR, SEM and EDS methods, underwent changes recorded by: (i) a reduction in Ca/P molar ratio in all the investigated materials resulting from modification in phase composition and (ii) weight increase in the investigated disc-samples, resulting from a precipitation of newly formed phase as an admixture of primary HPO_4^{2-} -free HAp. They were: secondary (with HPO_4^{2-} incorporated) HAp and chlorapatite in all the samples, while in addition to the listed above, β -tricalcium phosphate was formed on the discs made of HAp from stationary chamber oven, but not from rotary kiln. The new phases were formed in agglomerates on the surface of the investigated ceramic discs and in their pores. The results obtained proved that calcination conditions of pork bone sludge (temperature 750–950 °C, time and type of the applied kiln) under which hydroxyapatite (HAp) powders were obtained had an impact on its different behaviour in SBF, nevertheless, indicating a potential ability of the material to form a new biologically relevant interface with hard tissue and suitability for further investigations intended for medical grafting.

Keywords. Natural hydroxyapatite; simulated body fluid; incubation; biomaterial; pH changes; rotary calcining kiln; chamber oven.

1. Introduction

The subject of developing new biologically relevant materials useful for dentistry and skeletal tissue reconstruction on surgically operated patients have received a great deal of attention. Most of the materials are based on synthetic hydroxyapatite (HAp) and related calcium phosphates (Jarcho 1981; De Groot 1983; Hulbert 1990; Nishihara and Akagawa 1990; LeGeros 1991; Świąciki 1992; Knychalska-Karwan and Ślósarczyk 1994; LeGeros *et al* 1995a,b; Knychalska-Karwan *et al* 1998; Ducheyne and Qiu 1999; Benhayoune *et al* 2000; Orlovskii *et al* 2002; Li *et al* 2002; Raynaud *et al* 2002; Pena and Vallet-Regi 2003; Hing 2004; Hsu *et al* 2005; Li *et al* 2007; Sopyan *et al* 2007; Fathi *et al* 2008; Daculsi *et al* 2008; Brandt *et al* 2008; Salms *et al* 2009; Funayama *et al* 2009). Synthetic HAp-calcium phosphate of chemical and crystallographic similarity with mineral component of natural bone have an ability to form strong chemical bonds with the living hard tissue (Jarcho 1981; Nishihara and Akagawa 1990; Knychalska-Karwan *et al* 1998; Ducheyne and Qiu 1999; Benhayoune *et al* 2000; Fathi

et al 2008; Salms *et al* 2009; Funayama *et al* 2009). In bone-tissue engineering, porous HAp ceramics have found enormous use in biomedical applications including bone tissue regeneration, cell proliferation and drug delivery. It has been applied as a filler of bone defects, as a material for bone augmentation in prosthesis revision surgery and as a coating on implants such as total hip replacement (THR).

Synthetic hydroxyapatite with molar ratio of $\text{Ca/P} = 1.67$ with a corresponding stoichiometry of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ can be obtained hydrothermally above 300 °C or by solid-state reaction at temperatures above 900 °C (Kay *et al* 1964; LeGeros 1991). Stoichiometric hydroxyapatite is characterized by a monoclinic crystal structure (Kay *et al* 1964; LeGeros 1991). Synthetic hydroxyapatite precipitated from water solutions at room temperature and higher or by hydrolysis of acidic calcium phosphates is always non-stoichiometric, Ca-deficient, (def-HAp) is characterized by a molar ratio of Ca/P lower than 1.67 (Berry 1967; Posner *et al* 1984). The variation of Ca/P molar ratio is caused mostly by substitution of HPO_4^{2-} for phosphate PO_4^{3-} ion, while hydroxyapatite is formed in water solution (Posner *et al* 1980, 1984; LeGeros 1991). Synthetic-nonstoichiometric as well as biological apatites, constituents of bones and teeth are

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characterized by hexagonal crystal structure (LeGeros 1974, 1991; Posner *et al* 1980, 1984).

In hydroxyapatite structure, PO_4^{3-} anions can be partially substituted with carbonate group—type B of carbonated hydroxyapatite—as opposed to type A of high-temperature carbonated hydroxyapatite, in which hydroxyl groups are partially substituted with CO_3^{2-} (LeGeros *et al* 1968, 1971; Suetsugu *et al* 1997; Rajabi-Zamani *et al* 2008). Substitutions in anionic sublattice with other chemical elements such as chlorine or fluorine are common (LeGeros 1991; LeGeros *et al* 2006). Calcium partially substituted with magnesium (0.7 wt%), sodium (0.9 wt%), potassium (0.03 wt%) and microelements such as Sr, Pb, Zn, Cu and Fe have also been found (LeGeros *et al* 1989; 1995a, b; Veiderma *et al* 2005; Kalita and Bhatt 2007). The non-stoichiometry, particularly Ca-deficiency, as well as the presence of foreign ions, affect properties of hydroxyapatite such as reduction in crystal size (crystallinity), increase in solubility related to resorbability if the material has been implanted in human system, thermal stability is essential for elaboration of HAp based materials for further medical purpose, etc (Jarcho 1976; LeGeros 1991; Raynaud *et al* 1998, 2002; De Champos and Bressiani 2002; LeGeros *et al* 2006).

Fabrication of hydroxyapatite ceramics usually involves precipitation from water solution and subsequent sintering at temperatures higher than 1000 °C (Jarcho 1976; De Groot 1983; Raynaud *et al* 1998; De Champos and Bressiani 2002; Li *et al* 2002).

Before a precipitated Ca-deficient hydroxyapatite undergoes sintering and densification at temperatures above 1000 °C, several complex processes occur at lower temperature (Liao *et al* 1999; Gibson *et al* 2000; Wang and Dörner-Reisel 2004; Mostafa 2005; Shih *et al* 2006): (i) water adsorbed on the surface is released below 200 °C, (ii) temperature range of 300–500 °C, HPO_4^{2-} decomposition occurs according to reaction:

with a release of water proceeds and (iii) above 700 °C, partial dehydroxylation of hydroxyapatite is caused by the reaction:

At temperature higher than 1000 °C HAp gradually releases OH^- ions (Shih *et al* 2006). Sintering temperature higher than 1300 °C cause transformation:

and formation of tetracalcium phosphate (TTCP) = $\text{Ca}_4(\text{PO}_4)_2\text{O}$. If carbonate or foreign ions are incorporated into synthetic hydroxyapatite, thermal decomposition and transformation processes may be more (not less) complex. Thus, physicochemical properties (phase composition, specific surface area, density and mechanical properties)

of the sintered hydroxyapatite ceramics depend on the initial hydroxyapatite structure formed and the temperature of thermal treatment.

While synthetic HAp materials have been used widely in medical applications, attention has been focused recently on HAp preparation methods from animal bones supplied by slaughter-houses. It is believed that HAp from natural sources, after proper physicochemical treatment and thermal processing, can be regarded as an attractive material for further investigations intended for medical grafting (Rosen *et al* 2002; Murugan *et al* 2003; Goller *et al* 2006; Haberko *et al* 2006; Ooi *et al* 2007; Barakat *et al* 2009; Sobczak *et al* 2009, 2010a,b; Younesi *et al* 2010). The macroporosity of hydroxyapatite obtained from bovine bone is conserved to a greater or lesser extent, depending on the temperature of heat treatment, however, crystallinity of bone apatite is changing with temperature increase.

The ability of a material to form new bone on its surface (bioactivity), can be determined by *in vitro* experiments while the material is incubated in simulated body fluid (SBF). In the literature concerning hydroxyapatite, it has been claimed that the behaviour of the hydroxyapatite upon immersion in SBF depends on structure and composition of the material associated to some extent with a dissolution ability (Radin and Ducheyne 1993; Ducheyne *et al* 1993; Milev *et al* 2003). The hydroxyapatite dissolution rate is dependent mainly to the crystallinity level, phase composition, microstructure, surface area and density, etc (LeGeros 1991; Ducheyne *et al* 1993; LeGeros *et al* 1995a,b; Milev *et al* 2003). Some *in vitro* and *in vivo* investigations concerning nano-HA confirmed that Ca^{2+} ion releases from the synthetic hydroxyapatite powders are similar to bone apatite, however, significantly faster than microcrystalline HA (Murugan and Ramakrishna 2004; Mohammadi *et al* 2008).

Summing up, voluminous literature concerning hydroxyapatite biomaterials, selectively cited in the present paper, refers mostly to synthetic materials. Our research has been focused on biological properties of hydroxyapatite of natural origin obtained with the use of a process elaborated in our institute and described elsewhere (Sobczak *et al* 2009, 2010a,b; Sobczak-Kupiec and Wzorek 2012).

In the present work, hydroxyapatite was obtained from pork bone (called bone sludge) through two routes of calcining: (i) with the use of a typical laboratory stationary chamber oven and (ii) an enlarged laboratory scale rotary kiln heated by gas combustion. The rotary kiln (figure 1) used for calcining, was constructed at our request. The kiln tube was made of heat-resisting steel. Internal wall of the rotary kiln was covered with chamote ceramics. The bone sludge for calcining was fed into rotary kiln at the rate of 100 g·min⁻¹ concurrently with heating gas through a bucket (5) connected to a belt conveyor. Retention time of the material calcined in the kiln depends on rpm. Using 0.5 rpm, retention time was 40 min. The parameters for calcining were selected after some preliminary tests, including temperature calibration in the kiln. The temperature of the wall measured at the outlet of the tube was treated as a calcining temperature.

The present paper presents *in vitro* behaviour of hydroxyapatite obtained via two different methods with different set of physical parameters used for pork bone calcination in a stationary chamber or in a rotary kiln. In contrast to calcinations in the stationary chamber, the rotary kiln enabled agitation of the material calcined. The study aimed to investigate the influence of calcination parameters (temperature, holding time—differing according to type of the kiln) on the effects obtained after immersion of hydroxyapatite in simulated body fluid.

2. Experimental

2.1 Hydroxyapatite preparation

Deproteinized and defatted-bone pulp, received from meat plant, called bone sludge, was applied as a starting material in the present experiment. Bone sludge is a byproduct forming in a bone deproteinization process.

- (i) The first step of the process (preliminary calcining) was carried out at a temperature of 650 °C in chamber oven or in rotary kiln (figure 1). Calcining time in chamber oven was 3 h, while in the rotary kiln it was 40 min.

Figure 1. Picture of an experimentally enlarged laboratory scale rotary kiln. (a) Gas heater; (b) rotary heating tube of 1120 mm in length and 150 mm in diameter; (c) electronic controlling device; (d) after-burning chamber and (e) belt conveyor bucket.

- (ii) In the second stage of the process:

- (a) The material preliminarily calcined at 650 °C in the stationary chamber oven was re-calcined in the same chamber oven with electric heating at temperatures 750, 850 and 950 °C in air atmosphere for different times (2, 2.5 and 3 h).
(b) The material preliminarily calcined in the rotary kiln was re-calcined at three temperature measurements: 750, 850 and 950 °C recorded at the outlet of the rotary kiln with gaseous heating.

2.2 Assessment of *in vitro* bioactivity

Discs of a 13 mm diameter and weight amounting to 1.1 g of HAp powders obtained in the chamber (CH) as well as in the rotary (ROT) kiln were made by the uniaxial compaction without a binder in a steel mould at a pressure of 74 MPa. The compacts were heated with the rate of 10 deg·min⁻¹ up to 950 °C and sintered in air for 3 h.

The assessment of *in vitro* bioactivity was carried out in a solution of simulated body fluid (SBF) prepared according to Kokubo and Takadama (2006). SBF solution was related to a composition and concentration similar to that of human plasma. The immersion study was carried out at 37 °C by soaking the discs suspended vertically in a plastic container with 40 mL of SBF. The ion concentration of SBF (mmol) is given in table 1.

The ratio of the surface of the ceramic disc to SBF volume was 0.075 cm⁻¹. The time of soaking was 62 days. The solution was freshened up every 24 h for 7 days and then once a week. After the immersion time was completed, the discs were removed from the solution, washed with distilled water, dried at 105 °C and analysed. The solution pH was analysed and adjusted using the pH-meter (Elmetron) with a combined electrode (Elmetron). The result was a mean of three measurements.

2.3 Methods of phase analysis

The phase composition of the samples was analysed by X-ray diffraction method with the use of Philips X'Pert diffractometer equipped with a graphite monochromator PW 1752/00, CuK α 1.54 nm, Ni filter (40 kV, 30 mA).

Table 1. Comparison of ion concentration and pH value of SBF and human plasma (Kokubo and Takadama 2006).

	Ion concentration (mmol/dm ³)								pH
	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ₃ ²⁻	HPO ₄ ²⁻	SO ₄ ²⁻	
Human plasma	142.0	5.0	1.5	3.5	103.0	27.0	1.0	0.5	7.2–7.4
SBF	142.0	5.0	1.5	3.5	147.8	4.2	1.0	0.5	7.4

FT-IR infrared analyses, within the basic infrared range of 400–4000 cm^{-1} , were conducted with the use of Scimitar Series FTS 2000 spectrophotometer produced by the Digilab Co. The sample of 0.0007 g was pressed with 0.2000 ± 0.0011 g KBr into a pellet.

The molar ratio of Ca/P (before and after incubation) was determined by chemical analyses of HAp samples. Calcium was analysed by complexometry (EDTA in the presence of calcein and thymolphthalein as the indicator), while phosphorus as a P–Mo–V complex by colorimetry (Marcel Media).

The morphology and particle size of HA was observed by SEM–EDS, Hitachi.

3. Results and discussion

3.1 Characteristics of samples before soaking in SBF

Identification of powder diffraction patterns of the investigated samples was based on JCPDS–ICCD materials database. The d -spacing (related to angle of diffraction) and relative intensities of diffraction peaks observed corresponded to hydroxyapatite crystal structure (card JCPDS no. 9-432), while no other phase in the samples obtained was found. Typical XRD diffraction patterns of the two samples are presented in figure 2. All the samples obtained in stationary chamber as well as in rotary kiln exhibited (similar to figure 2) XRD patterns indicating hydroxyapatite hexagonal crystal structure.

FT-IR spectra (figure 3) demonstrate differences in crystal structure in relation to thermal treatment of the bone sludge. Spectrum (figure 3(a)) of non-thermally-treated bone sludge is typical of low crystallinity ‘apatitic’, as well as an amorphous phosphate (LeGeros *et al* 1973; LeGeros 1991). It is characterized by ‘loss of resolution in the characteristic PO_4^{3-}

Figure 2. X-ray diffraction patterns of hydroxyapatite obtained by calcining bone sludge; 2 h in stationary chamber oven at 950 °C and 40 min in rotary kiln at 950 °C.

bands (vibration modes ν_3 and ν_4) which are normally degenerate in the IR absorption spectra of apatites’ (LeGeros *et al* 1973; Fowler *et al* 1966). Absorption bands in the region of around 1400–1600 cm^{-1} wavenumber are showing the presence of carbonate group (CO_3^{2-}) and organic compound.

FT-IR spectrum (figure 3(b)), pre-ignited at 650 °C bone is typical of hydroxyapatite crystal structure characterized by OH group bands at wavenumber of ~ 610 cm^{-1} and ~ 3600 cm^{-1} (LeGeros *et al* 1973; LeGeros 1991). Absorption band at a wavenumber of ~ 960 cm^{-1} has been assigned to ν_1 -symmetric P–O stretching mode, at 1000–1100 cm^{-1} to ν_3 -asymmetrical stretching mode of P–O, while absorption bands recorded within the wavenumber range of 570–630 cm^{-1} were assigned to ν_4 -asymmetrical deformation of O–P–O vibrational mode in tetrahedral PO_4^{3-} ion. Thermal pre-treatment at 650 °C caused organic component burning out, elimination of most carbonate, while initial bone mineral component transformed into crystalline hydroxyapatite form.

Figure 3. FT-IR spectra of hydroxyapatite obtained by calcining of bone sludge: (a) as prepared, not calcined, (b) pre-calcined at 650 °C, (c) re-calcined at 950 °C in stationary chamber for 2 h and (d) re-calcined in rotary kiln (950 °C, 40 min).

FT-IR spectra (figure 3(c and d)) of the samples calcined at higher than 650 °C are characteristic of highly crystalline (high resolution of the bands) hydroxyapatite according to LeGeros *et al* (1973) and LeGeros (1991). However, spectra of the samples obtained in rotary kiln contained weak bands of CO_3^{2-} indicated in figure 3(c). The presence of some small amount of carbonate in the hydroxyapatite may have resulted from the presence of CO_2 in gaseous atmosphere in the rotary kiln heated by gas combustion.

3.2 Results obtained in SBF

Chemical stability and biological activity were evaluated on the basis of pH changes recorded with time of immersion in simulated body fluid (SBF)—relevant to natural human environment (figure 4).

When ceramic discs made of HAp produced in the chamber oven (CH) were incubated in simulated body fluid, an increase in pH value in the initial period of time was observed. During the first two days, the pH value of SBF reached the maximum and amounted to 7.67 and 7.81 for HA at 850 and 950 °C, respectively. On the third day of soaking, the pH value decreased to a level of ~ 7.50 . During the next

24 days, the pH value was varying insignificantly, however, a negligible tendency towards lower pH values at the end of incubation time was observed. The differences between pH of SBF in which HAp samples obtained from chamber oven at different temperatures: 750, 850 and 950 °C were not significant. It was also confirmed that the calcining time did not influence pH value of the SBF. In contrast, the samples made of HAp obtained in the rotary kiln (ROT) exhibited not only a distinct growth in pH value of SBF up to 8.03 (after 24 h) with a subsequent decrease and stabilization in the following days, a pH increase was also observed at the end of the immersion time and reached the final value amounting to 8.13 (figure 4). The behaviour of all HAp (ROT) discs in SBF was similar to each other and the calcining temperature exerted no significant influence on the environmental SBF pH. The observed pH increase in the initial period of immersion time with subsequent pH decrease in all the investigated samples may suggest that dissolution and precipitation processes in SBF were likely to occur (figure 4) (Raynaud *et al* 2002; Kim *et al* 2008). According to Kim *et al* (2008), two stages (dissolution/precipitation) in apatite formation are possibly occurring. Also, Ruixue *et al* (2006) reported initial Ca-concentration increase and subsequent decrease recorded with time of soaking in SBF. Thus, it can be presumed that at first stage of incubation some dissolution at the surface of HAp disc accompanied by pH increase in SBF leads to oversaturation, while in second stage, nucleation and re-precipitation of new hydroxyapatite proceeds.

The samples, after immersion in SBF, were collected, dried at a temperature of 105 °C to a constant weight and analysed. Figure 5 demonstrates that weight of all HAp discs increased during immersion time, suggesting continuous precipitation of a new solid. The most intensive weight increase was recorded in the discs made of HA calcined at 950 °C (CH), which after 62 days amounted to $\sim 3\%$ (figure 5); whereas the weight growth to a level of 2.3% was recorded for the samples made of HAp (CH) calcined at 750 and 850 °C. The smaller weight increase (up to 1.6%) was recorded in ceramic discs made of HAp obtained in the rotary

Figure 4. pH changes of simulated body fluid in course of HAp incubation.

Figure 5. Weight changes of HAp samples as a function of immersion time in SBF.

kiln. The general tendency towards weight increase during soaking in SBF can be related to a precipitation of calcium phosphate phases mainly on the surface and perhaps into the pores (Raynaud *et al* 2002; Medvecky *et al* 2006).

After chemical analysis, the content of calcium and phosphorus obtained were recalculated into Ca/P molar ratio (figure 6).

In all the investigated ceramic solids (from chamber and rotary kiln), the Ca/P molar ratio after *in vitro* test in simulated body fluid decreased in comparison with the Ca/P molar ratio before immersion. Ca/P molar ratio after immersion in SBF fluid was between 1.59 and 1.63. The observed Ca/P decrease resulted from precipitation of a newly formed Ca-deficient hydroxyapatite as well as some small amounts of other phosphates. According to Raynaud *et al* (1998, 2002), superficially deposited layer of Ca-deficient HAp, having HPO_4^- incorporated is as given below

Ca-deficiency coupled with HPO_4^{2-} incorporation has been known since 60th of XXst century when Berry (1967), later in 80th Posner *et al* (1980, 1984), LeGeros *et al* (1982) and LeGeros (1991) reported that HAp, precipitated from water solutions was Ca-deficient and contained internal hydrogen bonds between oxygen of adjacent orthophosphate groups, resulting in the presence of HPO_4^{2-} , not observed in the stoichiometric hydroxyapatite. Since then HPO_4^{2-} ion has always been referred to as the absorption band in IR spectra in synthetic as well as in some biological apatites.

Figure 7 representing a typical FT-IR spectrum after discs were soaking in SBF, gave an evidence that HAp containing HPO_4^{2-} appeared. The band of HPO_4^{2-} was found at a wavenumber of 870 cm^{-1} which was in agreement with the literature values. It has to be noted that HPO_4^{2-} band was not present in HAp material before soaking in SBF (compare

Figure 6. Comparison of Ca/P molar ratio in HA samples before and after immersion in SBF.

figure 7 with figure 3), because that was not precipitated from water solution. The rest of the spectrum in figure 7, except the absence of weak bands of the CO_3^{2-} groups, was similar to those before the immersion in SBF in figure 3.

XRD analysis of ceramic samples after soaking in SBF indicates some modification in phase composition—the appearance of different additional phases in relation to chamber/rotary kiln as recorded in figure 8. The main com-

Figure 7. Representative FT-IR spectrum of HAp ceramics obtained under the following conditions: 950 °C, 2 h, CH, after immersion in simulated body fluid.

Figure 8. Diffraction patterns of HA ceramics after soaking in simulated body fluid. Calcination parameters of HA powders (°C, h, kiln): (a) 750, 2, CH, (b) 750, 2.50, CH, (c) 750, 3, CH, (d) 850, 2, CH, (e) 850, 2.5, CH, (f) 850, 3, CH, (g) 950, 2, CH, (h) 950, 2.5, CH, (i) 950, 3, CH, (j) 750, ROT, (k) 850, ROT and (l) 950, ROT.

ponent is hydroxyapatite highlighted in the model X-ray spectrum in JCPDS 9-432. XRD peaks of low intensity according to JCPDS 33-271 corresponded to chlorapatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{Cl}_2$, appearing as an admixture in samples from both chamber and rotary kiln after SBF. In contrast, β variety of tricalcium phosphate (β -TCP) = β - $\text{Ca}_3(\text{PO}_4)_2$ highlighted by JCPDS 09-0169 appeared only in the samples calcined in chamber oven.

The microstructural characteristics of the HAp samples after 62-day immersion in SBF investigated by scanning electron microscopy are shown in figure 9. The observations are similar to those found in literature, for example, in Kim *et al* (2008). The precipitation of new phases on the surface of HAp ceramics as well as in the micropores was observed in samples obtained in chamber and also in rotary kiln. The precipitation of calcium phosphate phases on the surface of HAp samples obtained in the stationary oven was the most visible. Small spherical outgrowths of calcium phosphate agglomerates are formed on the surface of

HA bioceramic. In the case of ceramic discs made of HAp from the rotary kiln, the precipitation was less intensive and smaller aggregates were formed.

EDS analysis was performed in the samples after 62-day immersion in SBF. In all the cases, a similar spectrum was obtained. Figure 10 shows a representative EDS spectrum of HAp (950 °C, 2.5 h, CH). The highest number of counts in EDS spectrum of HAp samples after immersion in SBF corresponded to calcium and phosphorus—the main components of hydroxyapatite. The counts referred to carbon, sodium and oxygen are located within the range of the lowest energy 0.25–0.5 keV. The peak at 2.7 keV was assigned to chloride present in the chlorapatite structure.

Figure 11 presents the distribution of chemical elements on representative HAp sample surfaces after immersion in SBF. These images demonstrate a very good homogeneity of the analysed surfaces with one exception, the distribution of chlorine was not uniform. The distribution of chemical elements such as carbon, oxygen, phosphorus, calcium

Figure 9. SEM micrograph (1000× enlargement) of HA samples after 62-day immersion in SBF obtained under following conditions (temperature/time/kiln): (A) 750 °C, 2.5 h, CH, (B) 850 °C, 2.5, CH, (C) 950 °C, 2.5 h, CH, (D) 750 °C, ROT, (E) 850 °C, ROT and (F) 950 °C, ROT.

Figure 10. EDS spectrum of HA (950 °C, 2.5 h, CH) after 62-day of immersion in SBF at 37 °C.

Figure 11. Distribution of chemical elements on HA surfaces after immersion in SBF. (A) 750 °C, 2.5 h, CH, (B) 850 °C, 2.5 h, CH, (C) 950 °C, 2.5 h, CH and (D) 750 °C, 2.5 h, ROT.

and sodium in all the cases was almost uniform. The concentrations of these elements were located in an irregular manner on the surface of HAp samples, from both chamber and rotary kiln. A new chlorapatite phase was precipitated on the surface of HAp samples after immersion in simulated body fluid at 37 °C, however, this phase did not cover the whole surface.

4. Conclusions

In vitro (in SBF) behaviour of hydroxyapatite (HAp) obtained from pork bone sludge from meat plant via two essentially different calcination methods using a stationary, electrically heated chamber oven, and designed by the authors enlarged laboratory scale rotary kiln, heated by gas

combustion—methods enabling application of different set of physical parameters such as temperature, holding time—differing according to the type of the kiln can be summarized as below.

(I) HAp ceramic discs, after 62-day of incubation in SBF at 37 °C, characterized by X-ray diffraction (XRD), FTIR, SEM and EDS methods, underwent changes recorded by: (a) a reduction in Ca/P molar ratio in all the investigated materials resulting from modification in phase composition, (b) weight increase in the investigated disc samples, resulting from precipitation of newly formed phases, as an admixture of primary, HPO_4^{2-} -free HAp, (c) secondary (with HPO_4^{2-} incorporated) HAp and chlorapatite were formed on disc samples made of HAp calcined in chamber oven as well as in rotary kiln, (d) in addition to those listed in (c), β -tricalcium phosphate (β -TCP) was formed on the discs made of HAp from stationary chamber oven, but not from the rotary kiln and (e) the new phases were formed in agglomerates on the surface of the investigated ceramic discs and in their pores.

(II) The components of SBF as well as the constituents of primary hydroxyapatite (obtained in chamber oven or rotary kiln), possibly dissolving at the surface of the discs were the source of ions participating in phase composition modification after immersion in SBF. pH increase in the initial period of immersion time with subsequent pH decrease may suggest that dissolution and precipitation processes in SBF were likely to occur.

(III) The results obtained proved that calcination conditions of pork bone sludge (temperature 750–950 °C, time and type of the applied kiln) under which hydroxyapatite (HAp) powders were obtained had an impact on their different behaviours in SBF, nevertheless, indicating a potential ability of the material to form a new biologically relevant interface with hard tissue and suitability for further investigations intended for medical grafting.

The biologically relevant nature of the newly formed SBF deposits, containing a secondary HAp with HPO_4^{2-} incorporated, as well as β -TCP formed was confirmed by FT-IR (figure 7) and XRD (figure 8) results.

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