Acta of Bioengineering and Biomechanics
Vol. 15, No. 3, 2013

Original paper
DOI: 10.5277/abb130307

Physicochemical properties of the novel biphasic hydroxyapatite–magnesium phosphate biomaterial

DAWID PIJOCHA*, ANETA ZIMA, ZOFIA PASZKIEWICZ, ANNA ŚLÓSARCZYK

AGH – University of Science and Technology, Cracow, Poland.

Besides high-temperature calcium phosphates (CaPs), low-temperature calcium phosphate bone cements (CPCs), due to excellent biological properties: bioactivity, biocompability and osteoconductivity, are successfully used as bone substitutes. However, some disadvantages, related mainly to their low resorption rate and poor mechanical properties result in limited range of applications of these implant materials to non-loaded places in the skeletal system. To overcome this problem, magnesium phosphate cements (MPCs) with high strength have been considered as biomaterials. The main disadvantage of MPCs is that the acid-base setting reaction is an exothermic process that must be strictly controlled to avoid tissue necrosis.

In this work, a new composite bone substitute (Hydroxyapatite Magnesium Phosphate Material – HMPM) based on hydroxyapatite (HA) and magnesium phosphate cement (MPC) with sodium pyrophosphate applied as a retardant of setting reaction was obtained. Its setting time was adequate for clinical applications. Combining properties of HA and MPC has made it possible to obtain microporous (showing bimodal pore size distribution in the range of 0.005–1.700 micrometers) potential implant material showing good surgical handiness and sufficient mechanical strength. Effectiveness of sodium pyrophosphate as a retardant of exothermic setting reaction of the new cement formulation was confirmed. After setting and hardening, the material consisted of hydroxyapatite and struvite as crystalline phases. Unreacted magnesium oxide was not detected.

Key words: *composite, bone substitute, hydroxyapatite, magnesium phosphate cement*

1. Introduction

Calcium phosphates (HA, β-TCP, BCP) [1]–[5] and calcium phosphate cements (CPCs) [6]–[10] have been successfully applied in bone regenerative medicine for many years. First CPC materials were proposed in 1982 and 1983 by LeGeros [11] and Brown and Chow [12]. Their main advantages include: excellent biocompatibility, bioactivity and osteoconductivity. Besides that, CPCs are moldable and have an ability to set *in vivo* at ambient temperature. Currently there are a number of commercially available cements based on calcium phosphates for various applications. They are widely used in maxillofacial surgery [13], [14], for filling bone voids and defects in orthopaedy [15], as well as in vertebroplasty and arthroplasty [16]. Some attempts to use CPCs for fixation of metallic implants were also performed [17]. Not sufficiently high mechanical strength and rather poor reliability are their main disadvantages. Under *in vivo* conditions, due to low resorption rate, bone substitutes obtained by chemical bonding are slowly replaced by the newly formed bone. Thus, for medical applications faster bioresorption and new bone formation as well as better mechanical parameters are necessary. These requirements are met by magnesium phosphate cements (MPCs). MPCs were invented in the years of 1939–1941 as refractory materials used in stomatology for casting of metal alloys [18], [19]. At present the range of MPCs applications include mainly building engineering. Owing to such properties, as: very fast setting process and quick attainment of maximum mechanical strength they are suc-

* Corresponding author: Dawid Pijocha, AGH – University of Science and Technology, al. Mickiewicza 30, B6, r. 115, 30-059 Cracow, Poland. Tel: +48126175001, e-mail: dav_p@o2.pl

Received: January 30th, 2012
Accepted for publication: March 12th, 2013

cessfully applied as binding materials in road and airport runways repairs as well as the materials used to immobilize and store radioactive waste [20]. Mechanism of magnesium phosphate cement setting is based on an acid-base reaction between magnesium oxide (MgO) and phosphate ions originating from phosphoric acid or ammonium hydrophosphates – $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$ [21]. Crystalline struvite – $NH_4MgPO_4 \cdot 6H_2O$, as well as other magnesium phosphates, such as: $Mg_3(PO_4)_2 \cdot (0\text{-}4)H_2O$, $MgHPO_4$, $Mg(NH_4)_2H_2(PO_4)_2 \cdot 4H_2O$ in crystalline and amorphous forms are the MPC setting reaction products [21], [22]. The process occurs at room temperature, however, due to its exothermic nature, high amounts of heat are released during its course. Higher temperature of the reaction medium favors formation of amorphous $MgHPO_4$ and $MgHPO_4 \cdot 3H_2O$ [23]. Therefore MPC powder composition is modified in civil engineering applications of MPC by an addition of small amounts (2–10 wt. %) of such substances as: sodium borate, sodium pyrophosphate and calcium sulfates (CSH – $CaSO_4 \cdot 1/2H_2O$, CSD – $CaSO_4 \cdot 2H_2O$) which inhibit the setting process. Highly exothermic setting reaction is very dangerous in medical applications of biomaterials due to the necrosis of surrounding tissues. Kinetics of struvite crystal growth and, consequently, kinetics of MPC setting, depend mainly on surface properties of MgO, which are connected with the way of its preparation [24]. Bioactivity of MPCs is still investigated. Their main phase component, struvite, is a natural biomineral contained in, e.g., kidney stones [25]. Biocompatibility and biodegradation of MPC as well as lack of cytotoxicity of its degradation products formed under *in vivo* conditions have been confirmed by Wu et al. [26] and Yu et al. [27] They have shown that extracts of MPC do not cause DNA mutations, and hence they are not carcinogenic. Klammert et al. [28] found that struvite can be implanted without any negative reactions of a living body. Furthermore, MPC may exhibit antibacterial properties due to basic properties of its setting reaction products [29]. Combining the properties of CPC and MPC have allowed the development of biocompatible bone cement (Calcium–Magnesium Phosphate Cement) capable of stimulating osteogenesis *in vivo* and exhibiting very good mechanical properties [27].

In the present work, a new type of composite bone substitutes – HMPMs (Hydroxyapatite Magnesium Phosphate Materials) based on hydroxyapatite and magnesium phosphate cement have been developed. The novel HMPM formulations are characterized in terms of their acceptable for clinical applications setting times and well-controlled exothermic setting process. Moreover, other advantages of this new family of potential biomaterials are their phase compositions without unreacted MgO and microstructure.

2. Materials and methods

Initial HMPM powders were obtained by mixing MPC cement with HA powders (non-calcined or calcined) and sodium pyrophosphate – $Na_4P_2O_7 \cdot 10H_2O$ (Chempur, Poland) applied as the setting process retardant at various proportions (Table 1). In contrast to former studies, we used hydroxyapatite instead of calcium phosphate cement (CPC) composed of TTCP and DCPA commonly applied in calcium-magnesium phosphate cements preparation [26], [27]. MPC was prepared by mixing equimolar amounts of $NH_4H_2PO_4$ with MgO synthesized by calcination of basic magnesium carbonate $4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$ (POCH, Poland) at a temperature above 1100 °C. The powder obtained was sieved using the sieve of mesh size 0.063 mm. Crystalline $NH_4H_2PO_4$ (POCH, Poland) was ground in a mortar, then sieved (mesh size of a sieve: 0.1 mm). HA was obtained by the wet method using CaO and H_3PO_4 as reactants [4]. A part of HA prepared was calcined at 800 °C, then sieved (mesh size of a sieve: 0.1 mm). Surface area of the powders

Table 1. The initial cement formulations

Sample	HA non-calcined [wt. %]	HA calcined [wt. %]	MPC [wt. %]	Sodium pyrophosphate [wt. %]	HA/MPC weight ratio
HMPM 1	46	–	46	8	1:1
HMPM 2	57	–	37	6	3:2
HMPM 3	68	–	28	4	7:3
HMPM 1k	–	46	46	8	1:1
HMPM 2k	–	57	37	6	3:2
HMPM 3k	–	68	28	4	7:3

Table 2. Specific surface area of initial components

Component	BET specific surface area [m^2/g]
MgO	3.66
$NH_4H_2PO_4$	0.87
Hydroxyapatite – non-calcined	96.50
Hydroxyapatite – calcined	18.27

was measured by BET method. The results are presented in Table 2.

The self-setting cement-type composite samples were prepared by mixing powder batches with distilled water as a liquid phase at the L/P ratios in the range of 0.4–0.7 for achieving appropriate rheological consistency. Setting times were determined using the Gillmore testing apparatus. Phase compositions of the samples 24 hours after setting and hardening were established by X-ray diffraction in the 2θ angle range of 10°–90°. Quantitative phase compositions were calculated by the Rietveld method. Porosity as well as the pore size distributions after hardening were determined by mercury porosimetry (Autopore IV porosimeter of Micromeritics company). Microstructure was investigated by scanning electron microscopy (Nova 200 Nanosem of FEI Co. microscope) at magnifications of 2000, 5000 and 10000. Compressive strength of the batch of 10 cylindrical samples (diameter: 6 mm, height: 12 mm) was determined using Instron 3345 instrument with 2 mm/s cross-head speed. Samples for mechanical investigations were shaped manually in order to reflect the conditions during surgery when implant materials are prepared. Chemical stability of the materials under study was evaluated *in vitro* by measuring pH and ionic conductivity vs. the time of incubation of the samples in SBF and distilled water (pH-meter Hanna H198129 Combo). The cylindrical samples (12 mm in diameter and 4 mm in height) were prepared and after 60 min of hardening were placed into the container with 40 mL of SBF or distilled water and stored at 37 °C for 60 days. SBF solution was prepared according to Kokubo [30]. After 7, 14, 30 and 60 days of soaking in SBF, the samples were gently rinsed with distilled water to remove SBF solution followed by drying at 50 °C. Surfaces of the samples were characterized by SEM (magnification 5000× and 20000×) and EDS for *in vitro* evaluation of bioactivity.

3. Results

The initial and final setting times for the individual compositions have been in the range between 4–5 min and 9–10 min for the samples based on non-calcined HA and 6–8 min and 12–15 min for the samples based on calcined HA. Setting times as well as L/P ratio for the series of HMPM samples are collected in Table 3.

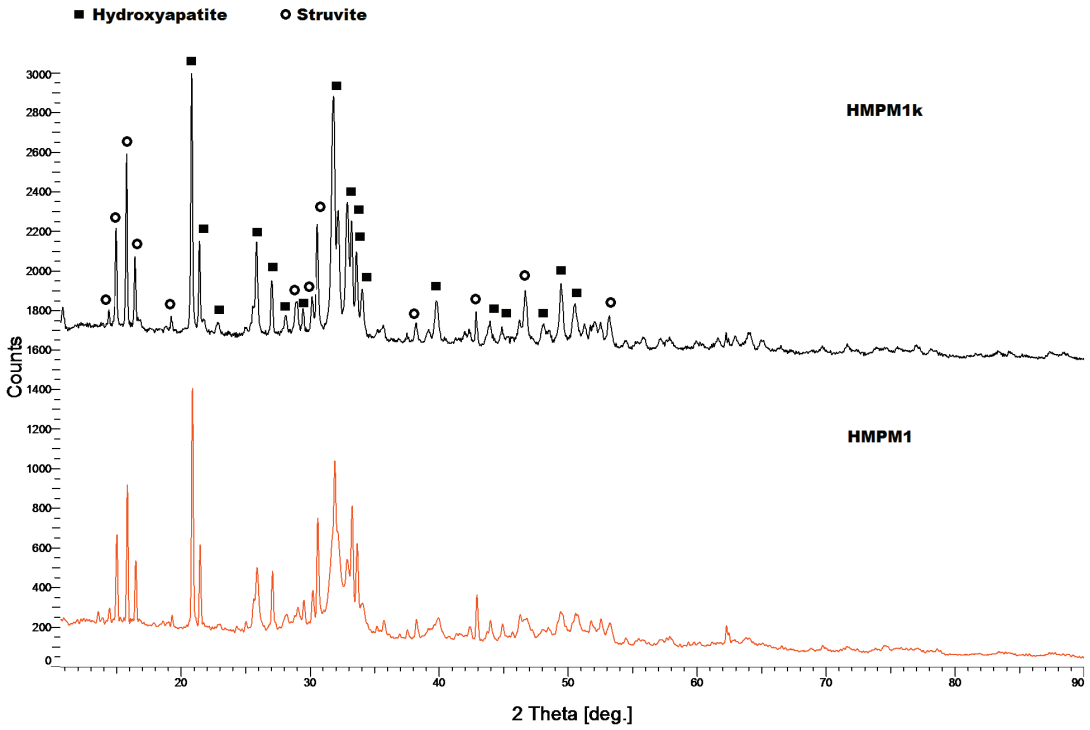

Fig. 1. XRD patterns of HMPM1 and HMPM 1k materials after setting and hardening for 24 hours

Table 3. Setting times and L/P ratios for the material obtained

Sample	L/P [ml/g]	Initial setting time t_0 [min]	Final setting time t_f [min]
HMPM 1	0.4	4	9
HMPM 2	0.5	5	10
HMPM 3	0.6	4	9
HMPM 1k	0.5	8	15
HMPM 2k	0.6	7	14
HMPM 3k	0.7	6	12

Table 4. Phase compositions of the HMPM samples after setting and hardening

Sample	HA [wt. %]	Struvite [wt. %]
HMPM 1	47.4	52.6
HMPM 2	53.6	46.4
HMPM 3	66.2	33.8
HMPM 1k	52.0	48.0
HMPM 2k	53.6	46.4
HMPM 3k	56.0	44.0

XRD investigations have shown that HA and struvite are the only crystalline phases present in the hardened samples (Table 4). XRD patterns of HMPM1 and HMPM1k materials are shown in Fig. 1. Values reported in Table 4 correspond well to the amounts of initial HA and MPC used for materials preparation.

Open porosity as well as pore size distribution values of the HMPM materials are given in Table 5. Open porosity has been in the range of 18.4–51.3% and depended significantly on the HA/MPC ratio in the solid phase compositions. Pore size distributions

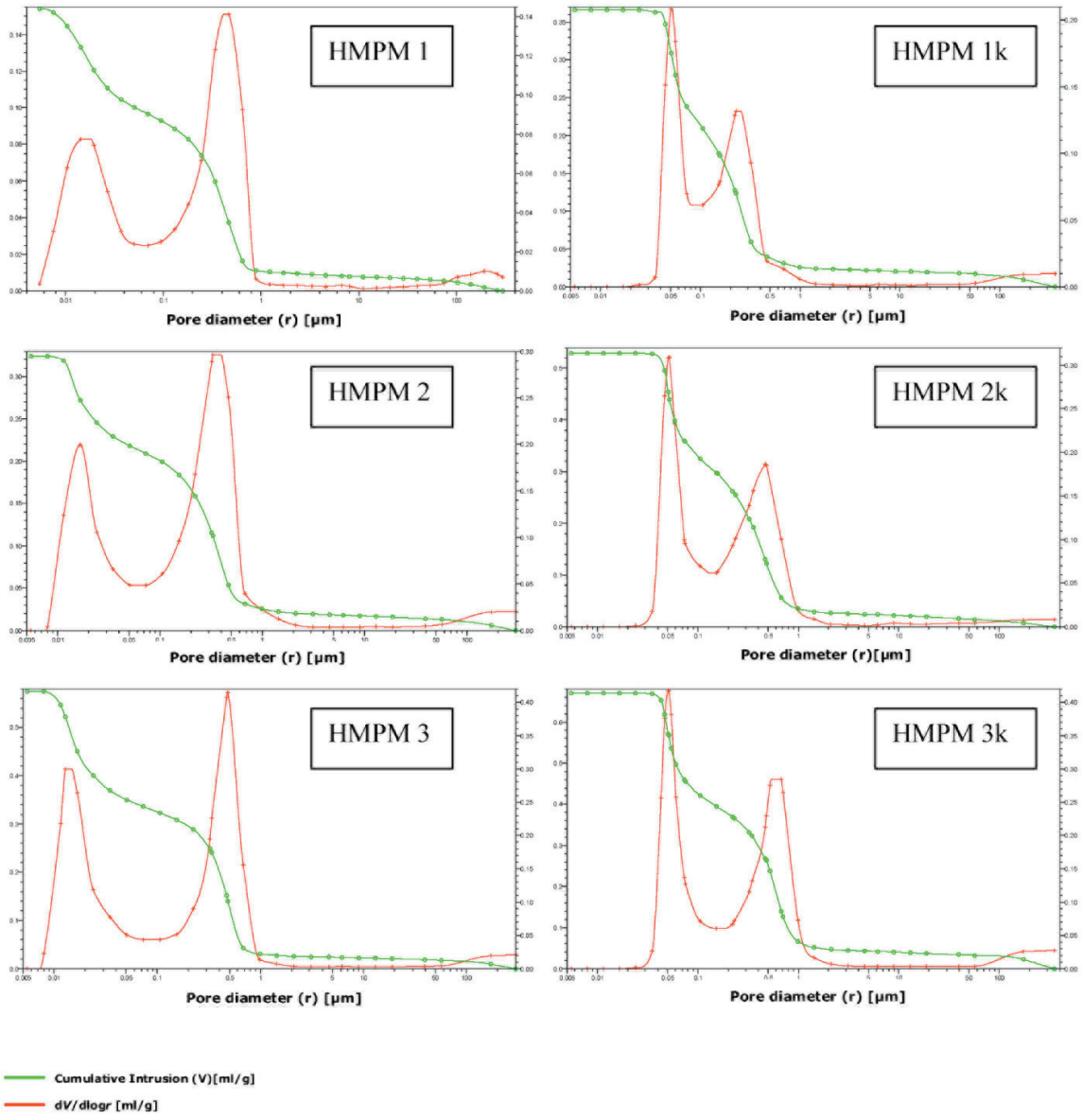

Fig. 2. Pore size distributions of the HMPM samples after setting and hardening

have been bimodal in the range of 0.006 μm to 1.700 μm (Fig. 2).

Table 5. Open porosity and pore size ranges of HMPM samples after setting and hardening

Sample	Open porosity [%]	Pore diameter [μm]	
		Range I	Range II
HMPM 1	18.4	0.006–0.060 (max. 0.016)	0.060–1.100 (max. 0.260)
HMPM 2	39.5	0.009–0.062 (max. 0.018)	0.062–1.700 (max. 0.244)
HMPM 3	41.2	0.005–0.039 (max. 0.015)	0.039–0,810 (max. 0.120)
HMPM 1k	31.1	0.020–0.091 (max. 0.051)	0.091–1.100 (max. 0.240)
HMPM 2k	40.7	0.020–0.130 (max. 0.051)	0.130–1.700 (max. 0.470)
HMPM 3k	51.3	0.024–0.160 (max. 0.050)	0.160–1.700 (max. 0.610)

Compressive strength of HMPM materials showed good correlation with their porosity: higher open porosity has led to lower compressive strength. Samples containing higher amounts of struvite have exhibited higher compressive strength in the case of both non-calcined and calcined HA-based formulations (Fig. 3). HA calcination increases compressive strength of HMPM materials only to a limited extent. The highest values, namely 17.3 ± 2.2 MPa and 17.8 ± 4.4 MPa, have been recorded for HMPM1 and HMPM1k samples, respectively, whereas the lowest – for the samples showing the highest open porosities, i.e., HMPM3 and HMPM3k (their values have been as low as 2.4 ± 0.6 MPa and 6.0 ± 1.3 MPa).

SEM investigations of the fracture surfaces of all the samples 24 hours after setting were conducted (Fig. 4). SEM observations of HMPM1 composite after 30 days of holding it at 37 °C in an inert atmosphere were also performed (Fig. 5).

Results of the studies of distilled water as well as SBF solution after incubation of the samples are presented in Figs. 6 and 7. Abrupt increase in conductivity of distilled water during the first 4–5 days of HMPM materials soaking (up to 880 μS on the 4th day for the HMPM3k material, and even up to 2280 μS for the HMPM1 material) is seen. This increase grows as the content of struvite in the material increases. Calcination of hydroxyapatite resulted in the distinct decrease in solubility of the composite. After this time, a slight lowering in conductivity of distilled water could be observed in the case of all samples until ca. 30th day of incubation, and then this parameter slowly stabilized. After 60 days, conductivity reached the values of 610 μS and 1830 μS for the HMPM3k and HMPM1 materials, respectively.

Chemical stability studies conducted during 60 days of incubation of the samples in SBF solution showed only a low increase in pH – to the value of 7.67 (for HMPM2k and HMPM3k materials) and to 7.87 (for HMPM1 material). The increase was higher for the materials with greater struvite content and for those based on non-calcined hydroxyapatite. Thus, the materials developed are chemically stable. SEM images of the surface of HMPM1 material after soaking in SBF are presented in Figs. 8–11. Growth of apatite layer was confirmed by EDS investigations. Additionally, the growth of struvite crystals during incubation as well as the presence of small calcium phosphate deposits on the surface of these crystals after 30 and 60 days of incubation were observed (Figs. 10, 11).

4. Discussion

Hydroxyapatite and struvite-based cement-type composite showing acceptable values of setting times, from 4 to 15 min (Table 3), was obtained. Such values are optimal for proper application of bone cement into the defect and decide on its high surgical handiness [31]. The setting of the materials developed begins within ca. 3–5 min after the addition of the liquid to the powder (the so-called "lag time") and depends on the type of hydroxyaptite applied. This is connected with the mechanism of MgO dissolution. Cement setting process involves combination of 5 water molecules with 1 molecule of magnesium oxide and ammonium dihydrogen phosphate (equation (1)) and proceeds step-wise. At first, after addition of the liquid, dihydrogen phosphate, as the most soluble component, is dissolved. This results in the lowering of pH of the system and dissolution of magnesium oxide with release of OH^- ions. Owing to this, pH gradually increases to neutral value. The higher acidity of the solution, the more MgO is dissolved. This leads to the increase in the yield of the reaction of struvite formation. In the present work, it has been decided to use equimolar amounts of reagents in order to avoid remaining of unreacted MgO in the system. Formation of crystalline lattice of struvite from $[Mg(H_2O)_6]^{2+}$ complexes, NH_4^+ and PO_4^{3-} ions present in the solution is the final stage of the process. Reaction stops when there are no more starting compounds left in the solution.

$$NH_4H_2PO_4 + MgO + 5H_2O = NH_4MgPO_4 \cdot 6H_2O \quad (1)$$

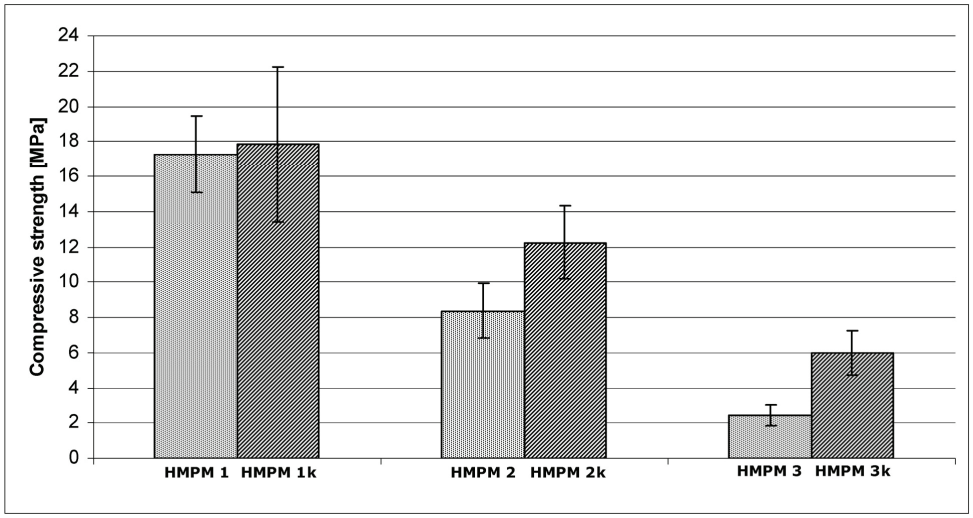

Fig. 3. Compressive strength of the HMPM samples, 24h after setting.
Dotted bars – materials based on non-calcined HA, striped bars – materials based on calcined HA

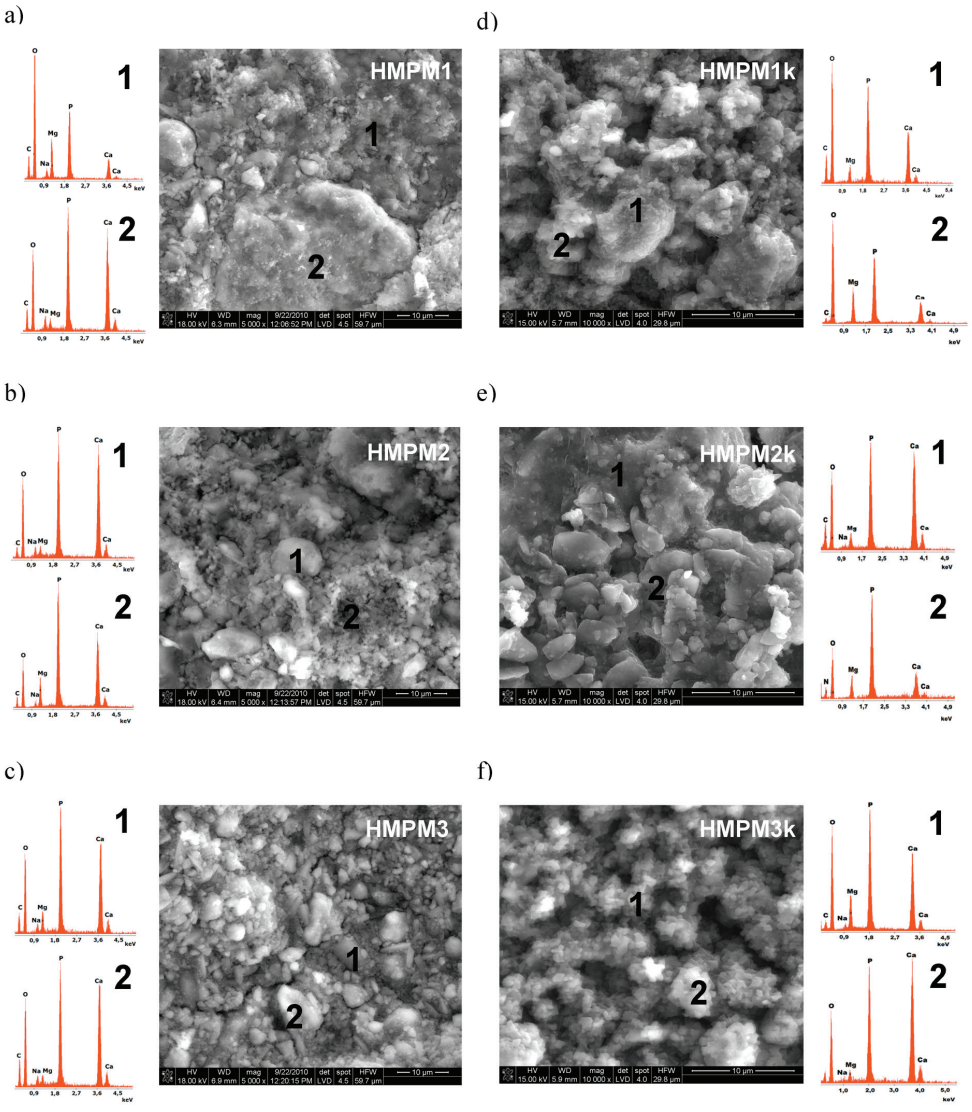

Fig. 4. Microstructure and EDS analyses of HMPM samples (fracture surfaces, 24 h after setting):
(a)–(c) materials based on non-calcined HA, (d)–(e): materials based on calcined HA

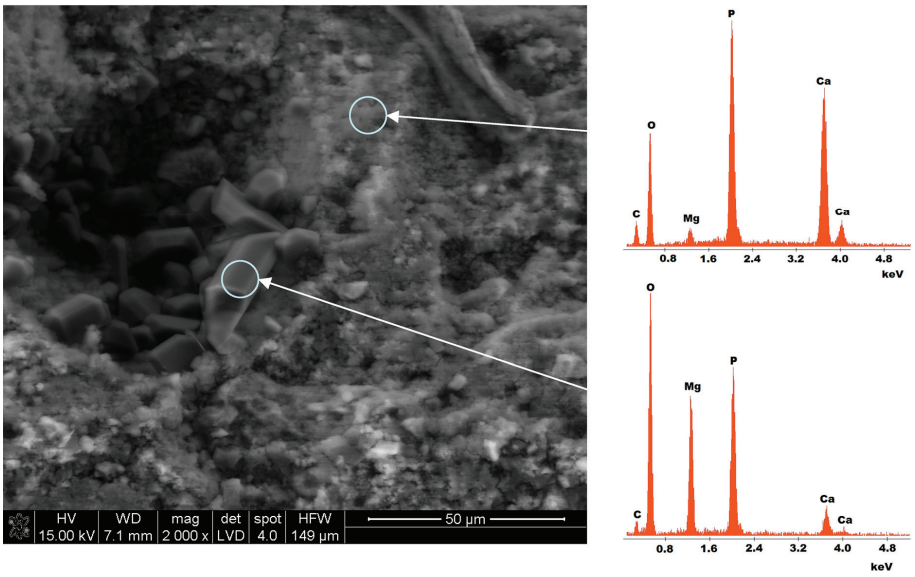

Fig. 5. Microstructure and EDS analysis of HMPM 1 sample
(fracture surface after 30 days of holding at 37 °C, inert atmosphere)

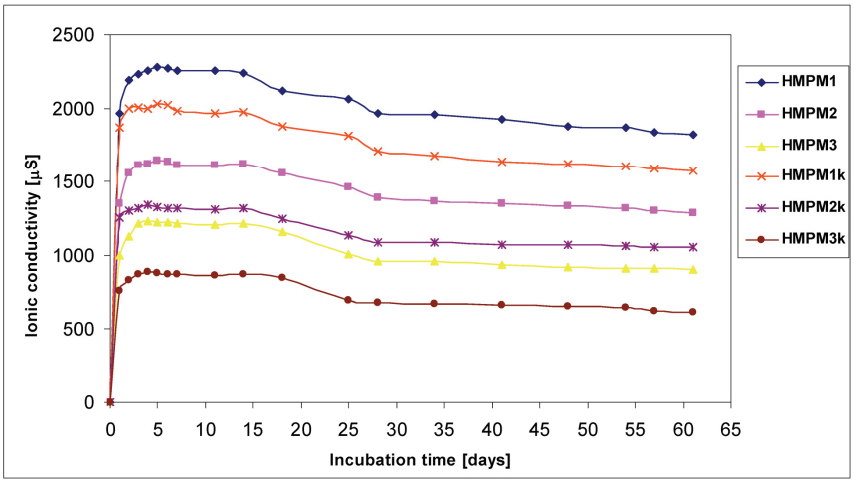

Fig. 6. Ionic conductivity of the HMPM samples after 60 days of incubation

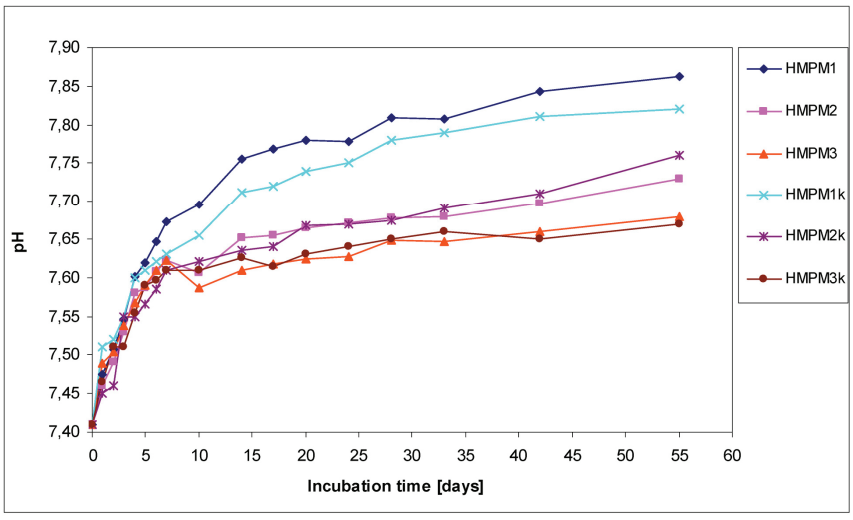

Fig. 7. pH of the SBF solution after 60 days of incubation

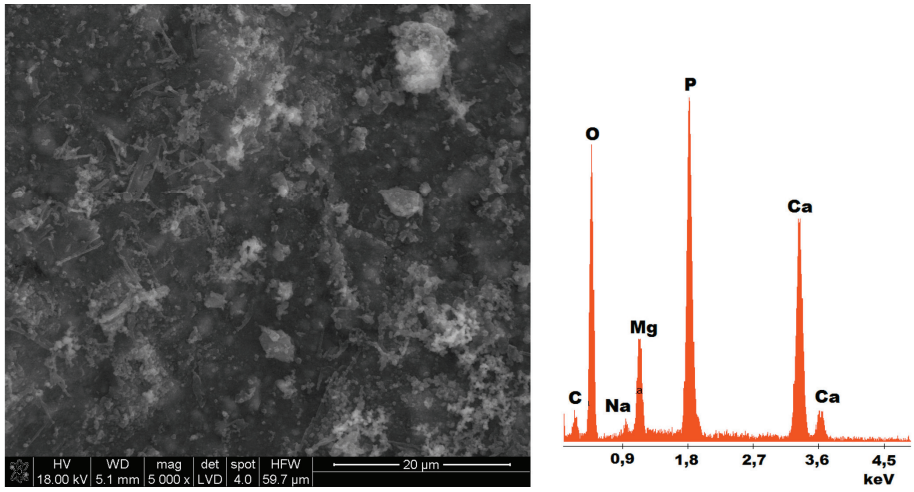

Fig. 8. SEM micrograph and average EDS analysis of the surface of the HMPM1 material 7 days after incubation in SBF (EDS spectra applied to the whole surface of the picture)

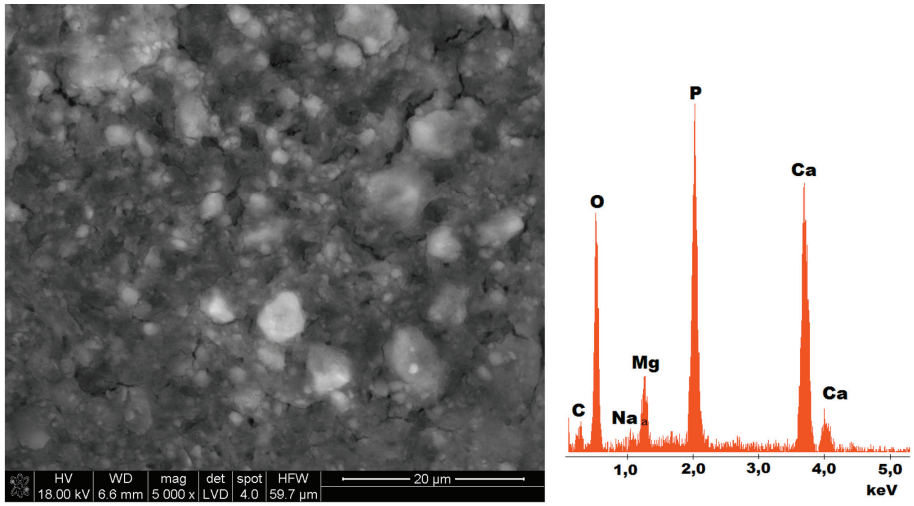

Fig. 9. SEM micrograph and average EDS analysis of the surface of the HMPM1 material 14 days after incubation in SBF (EDS spectra applied to the whole surface of the picture)

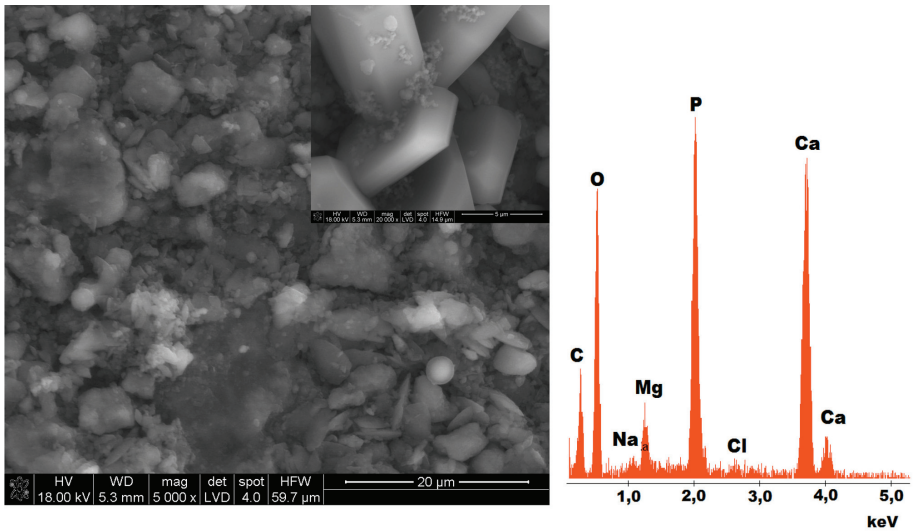

Fig. 10. SEM micrograph and average EDS analysis of the surface of the HMPM1 material 30 days after incubation in SBF (EDS spectra applied to the whole surface of the picture)

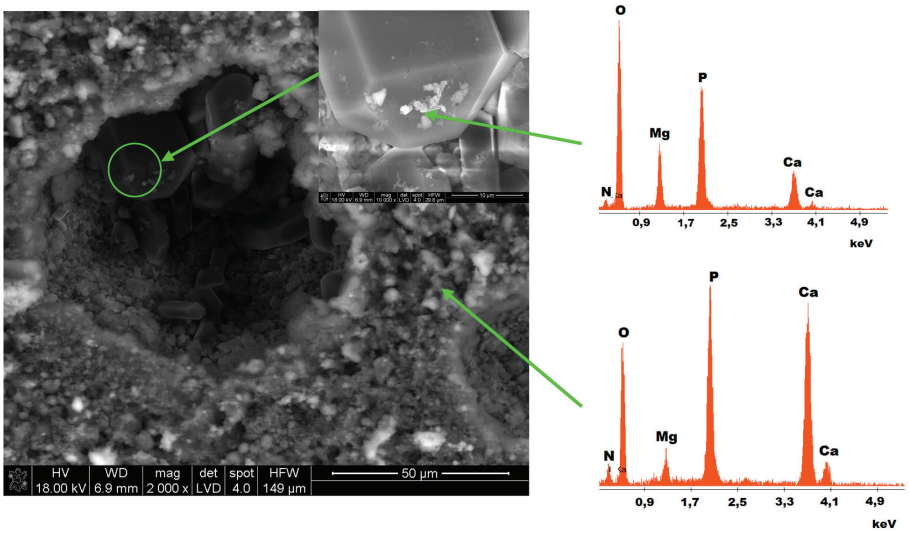

Fig. 11. SEM micrograph and EDS analysis of the surface of the HMPM1 material 60 days after incubation in SBF

Sodium pyrophosphate has been proved to be an efficient retardant of the setting process, also capable of lowering its temperature. Thermal effects occurring during the setting of HMPM material were studied earlier [32]. They showed that both calcium sulfate hemihydrate (CSH) and sodium pyrophosphate are efficient retardants of the setting reaction that also lower the high reaction temperature. The present studies, in which exclusively $Na_4P_2O_7{\cdot}10H_2O$ was applied, have confirmed that it is possible to use one compound as the retardant. This component can replace sodium borate in MPC cements [26], [29]. The maximum temperature during the setting of HMPM composite was equal to 50 °C.

Materials containing two crystalline phases: hydroxyapatite (47.4 %–66.2 wt. %) and struvite (33.8–52.6 wt. %) were obtained. In the composite developed, non-calcined, subjected only to preliminary thermal treatment hydroxyapatite served as the source of CaPs which – after setting of the material – preserved its crystalline form. In the CPC-type materials described in the literature, hydroxyapatite resulted from hydrolysis of various calcium phosphates, e.g., TTCP, DCPA [26], [27]. In contrast to the results obtained by other authors [26], [29], among the phases present in our composite no unreacted magnesium oxide was detected. Magnesium oxide present in the final setting product may cause high, local increase in pH around the material placed in the bone defect, which is disadvantageous for cells and tissue.

The influence of hydroxyapatite thermal treatment on the porosity and mechanical strength of the materials formed after setting has been observed. Porosimetric investigations have shown that with the increase in MPC content in the initial formulation, a decrease in open porosity of the hardened materials has occurred for both types of materials, i.e., based on non-calcined and calcined HA. Shift in the position of the first maximum on the $d\log V/dr = f(r)$ curves was observed in the case of samples based on calcined HA. These samples have given a distinct maximum (ca. 0.050 µm) and have exhibited a narrow pore size distribution. In contrast, for the samples based on non-calcined HA – the first range of pore sizes was broader and the extreme value was shifted to lower pore sizes – from 0.018 to 0.015 µm (Fig. 2).

Results of mechanical strength measurements have confirmed that less porous materials based on calcined HA show higher compressive strength than those based on non-calcined HA. The composites prepared in the work exhibit higher mechanical strength than some CPC cements [33]–[35], lower, however, than other CMPC composites [26]. This may be due to various methods of their synthesis. Differences in porosities between the composites based on non-calcined and calcined HA are clearly visible in SEM images of surface fractures of HMPM1-3 and HMPM1k-3k samples (Fig. 4). Around HA grains, magnesium phosphate is formed, which has been confirmed by EDS investigations. In the course of incubation of the samples at a temperature of 37 °C in the atmosphere of high humidity struvite crystals grow. Thick, romboidal crystals of struvite [36]–[38] grow in the voids inside the material (Fig. 5). Chemical stablity tests in distilled water conducted *in vitro* prove the deposition of calcium phosphates [35] or magnesium phosphates (growth of struvite crystals due to recrystallization in distilled water) on the surfaces of the samples.

The developed hydroxyapatite–struvite composite incubated in SBF solution shows bioactivity. During

a 60 day incubation, growth of struvite crystals has been observed, particularly clearly distinguishable in SEM micrographs recorded after 30 and 60 days (Figs. 10 and 11). Presence of small amounts of apatite deposits on the surface of large crystals has been detected. Struvite crystals have been seen first in the open pores on the surface of the samples. Positive results of *in vivo* tests conducted by Wu et al. [26] and Yu et al. [27] for hardened CPC and MPC-based cements imply that the HMPM material studied may show good properties in further biological studies.

5. Concluding remarks

Microporous chemically bonded ceramics (CBCs) based on calcium and magnesium phosphates have been obtained. The material developed in the form of moldable paste is easy to prepare and to fill the bone defects. After setting and hardening it shows porosity varying from 18 to 51% and bimodal pore size distribution in the range of 0.005–1.700 μm. The new HMPM bone substitute contains, depending on initial raw materials, two crystalline phases: HA and struvite ($NH_4MgPO_4 \cdot 6H_2O$) without the presence of unreacted MgO. Effectiveness of sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) as a retardant of exothermic setting reaction was confirmed. Thermal effects of setting reaction were successfully limited. The setting times within 4–15 minutes were sufficient for bone-filling materials to be placed in the site of implantation. The positive influence of struvite on mechanical strength of final composites was found. Further evaluation of developed materials in biological tests is recommended. Combined CPCs–MPCs materials were shown to be biocompatible and osteogenic *in vivo* [26], [29], [34].

Acknowledgements

This work was supported by the European Union – project No. UDA-POIG-01.03.01-00-005/09.

References

[1] BLITTERSWIJK C.V. (ed.), *Tissue Engineering, Academic Press Series in Biomedical Engineering*, Chapter 8, Elsevier, Amsterdam, 2008.

[2] DETSCH R., MAYR H., ZIEGLER G., *Formation of osteoclast-like cells on HA and TCP ceramics*, Acta Biomater., 2008, Vol. 4, 139–148.

[3] LI B., CHEN X., GUO B., WANG X., FAN H., ZHANG X., *Fabrication and cellular biocompatibility of porous carbonated biphasic calcium phosphate ceramics with a nanostructure*, Acta Biomater., 2009, Vol. 5, 134–143.

[4] ŚLÓSARCZYK A., STOBIERSKA E., PASZKIEWICZ Z., GAWLICKI M., *Calcium Phosphate Materials Prepared from Precipitates with Various Calcium: Phosphorus Molar Ratios*, J. Am. Ceram. Soc., 1996, Vol. 79(10), 2539–2544.

[5] ZHU Z.L., YU H.Y., ZENG Q., HE H.W., *Characterization and biocompatibility of fluoridated biphasic calcium phosphate ceramics*, Appl. Surf. Sci., 2008, Vol. 255, 552–554.

[6] APELT D., THEISS F., EL-WARRAK A.O., ZLINSZKY K., BETTSCHART-WOLFISBERGER R., BOHNER M., MATTER S., AUER J.A., von RECHENBERG B., *In vivo behavior of three different injectable hydraulic calcium phosphate cements*, Biomaterials, 2004, Vol. 25, 1439–1451.

[7] FERNANDEZ E., GIL F.J., GINEBRA M.P., DRIESSENS F.C. M., PLANELL J.A., BES S.M., *Production and characterization of new calcium phosphate bone cements in the $CaHPO_4$ – α-$Ca_3(PO_4)_2$ system: pH, workability and setting times*, J. Mater. Sci. – Mater. Med., 1999, Vol. 10, 223–230.

[8] FERNANDEZ E., GINEBRA M.P., BERMUDEZ O., BOLTONG M.G., DRIESSENS F.C.M., PLANELL J.A., *Dimensional and thermal behaviour of calcium phosphates cements during setting compared to PMMA bone cements*, J. Mater. Sci. Lett., 1995, Vol. 14, 4–5.

[9] GINEBRA M.P., CANAL C., ESPANOL M., PASTORINO D., MONTUFAR E.B., *Calcium phosphate cements as drug delivery materials*, Adv. Drug Del. Reviews, 2010, Vol. 64(12), 1090–1110.

[10] LOPEZ-HEREDIA M.A., BONGIO M., BOHNER M., CUIJPERS V., WINNUBST L.A.J.A, DIJK N., WOLKE J.G. C., BEUCKEN J.J.J.P., JANSEN J.A., *Processing and in vivo evaluation of multiphasic calcium phosphate cements with dual tricalcium phosphate phases*, Acta Biomater., 2012, Vol. 8(9), 3500–3508.

[11] LeGEROS R., CHOHAYEB A., SHULMAN A., *Apatitic calcium phosphates: possible dental restorative materials*, J. Dent. Res., 1982, Vol. 61, 343.

[12] BROWN W.E., CHOW L.C., *A new calcium phosphate setting cement*, J. Dent. Res., 1983, Vol. 62, 672.

[13] WOLFF K.D., SWAID S., NOLTE D., BÖCKMANN RA., HÖLZLE F., MÜLLER-MAI C., *Degradable injectable bone cement in maxillofacialsurgery: indications and clinical experience in 27 patients*, J. Cranio. Maxill. Surg., 2004, Vol. 32(2), 71–79.

[14] KLAMMERT U., GBURECK U., VORNDRAN E., RÖDIGER J., MEYER-MARCOTTY P., KÜBLER A.C., *3D powder printed calcium phosphate implants for reconstruction of cranial and maxillofacial defects*, Original Research Article, J. Cranio. Maxill. Surg., 2010, Vol. 38(8), 565–570.

[15] JACOFSKY D.J., MCCAMLEY J.D, JACZYNSKI A.M., SHRADER M.W., JACOFSKY M.C., *Improving Initial Acetabular Component Stability in Revision Total Hip Arthroplasty : Calcium Phosphate Cement vs Reverse Reamed Cancellous Allograft*, J. Arthorplasty, 2012, Vol. 27(2), 305–309.

[16] LEWIS G., *Injectable bone cements for use in vertebroplasty and kyphoplasty: state-of-the-art review*, J. Biomed. Mater. Res. B Appl. Biomater., 2006, Vol. 76, 456–468.

[17] OOMS E., WOLKE J., van der WAERDEN J., JANSEN J., *Use of injectable calcium-phosphate cement for the fixation of titanium implants: an experimental study in goats*, J. Biomed. Mater. Res. B Appl. Biomater., 2003, Vol. 66, 447–456.

[18] PROSEN E.M., *Refractory material suitable for use in casting dental investments*, US Patent No. 2209404 (1941).

[19] PROSEN E.M., *Refractory materials for use in making dental casting*, US Patent No. 2152152 (1939).

[20] SEEHRA S.S., GUPTA S., KUMAR S.S., *Rapid setting magnesium phosphate cement for quick repair of concrete pavements – characterization and durability aspects*, Cement Concrete Res., 1993, Vol. 23, 254–266.

[21] SOUDEE E., PERA J., *Influence of magnesia surface on the setting time of magnesia–phosphate cement*, Cement Concrete Res., 2002, Vol. 32, 153–157.

[22] GILHAM-DAYTON P.A., *The phosphate bonding of refractory materials*, Brit. Ceram. Trans. J., 1963, Vol. 62, 895–904.

[23] SCRIMGEOUR S.N., CHUDEK J.A., COWPERA G.A., LLOYDA C.H., *31P solid-state MAS-NMR spectroscopy of the compounds that form in phosphate-bonded dental casting investment materials during setting*, Dent. Mater., 2007, Vol. 23, 934–943.

[24] SOUDEE E., PERA J., *Mechanism of setting reaction in magnesia-phosphate cements*, Cement Concrete Res., 2000, Vol. 30, 315–321.

[25] TAMIMI F., NIHOUANNEN D.L., BASSETT D.C., IBASCO S., GBURECK U., KNOWLES J., WRIGHT A., FLYNN A., KOMAROVA S.V., BARRALET J.E., *Biocompatibility of magnesium phosphate minerals and their stability under physiological conditions*, Acta Biomater., 2011, Vol. 7, 2678–2685.

[26] WU F., WEI J., GUO H., CHEN F., HONG H., LIU C., *Self-setting bioactive calcium–magnesium phosphate cement with high strength and degradability for bone regeneration*, Acta Biomater., 2008, Vol. 4, 1873–1884.

[27] YU Y., WANG J., LIU C., ZHANG B., CHEN H., GUO H., ZHONG G., QU W., JIANG S., HUANG H., *Evaluation of inherent toxicology and biocompatibility of magnesium phosphate bone cement*, Colloids Surf. B Biointerfaces, 2010, Vol. 76, 496–504.

[28] KLAMMERT U., IGNATIUS A., WOLFRAM U., REUTHER T., GBURECK U., *In vivo degradation of low temperature calcium and magnesium phosphate ceramics in a heterotopic model*, Acta Biomater., 2011, Vol. 7(9), 3469–3475.

[29] MESTRES G., GINEBRA M.P., *Novel magnesium phosphate cements with high early strength and antibacterial properties*, Acta Biomater., 2011, Vol. 7, 1853–1861.

[30] KOKUBO T., TAKADAMA H., *How useful is SBF in predicting in vivo bone bioactivity?*, Biomaterials, 2006, Vol. 27, 2907–2915.

[31] DEB S. (ed.), *Orthopaedic bone cements*, [in:] M.P. Ginebra, *Calcium Phosphate Cements*, Woodhead Publishing Limited, England, 2008.

[32] PIJOCHA D., ŁÓJ G., NOCUŃ-WCZELIK W., ŚLÓSARCZYK A., *Effect of retardants on the heat release during setting of bone cement-type composites*, Journal of Achievements in Materials and Manufacturing Engineering, 2011, Vol. 49(2), 204–209.

[33] WEI J., JIA J., WU F., WEI S., ZHOU H., ZHANG H., SHIN J.W., LIU C., *Hierarchically microporous/macroporous scaffold of magnesium-calcium phosphate for bone tissue regeneration*, Biomaterials, 2010, Vol. 31, 1260–1269.

[34] KIM S.Y., JEON S.H., *Setting properties, mechanical strength and in vivo evaluation of calcium phosphate-based bone cements*, J. Ind. Eng. Chem., 2012, Vol. 18, 128–136.

[35] ZIMA A., PASZKIEWICZ Z., SIEK D., CZECHOWSKA J., ŚLÓSARCZYK A., *Study on the new bone cement based on calcium sulfate and Mg, CO3 doped hydroxyapatite*, Ceram. Int., 2012, Vol. 38, 4935–4942.

[36] DOYLE J.D., PARSONS S.A., *Struvite formation, control and recovery*, Water Research, 2002, Vol. 36, 3925–3940.

[37] DOYLE J.D., PHILP R., CHURCHLEY J., PARSONS J.A., *Analysis of struvite precipitation in real and synthetic liquors*, Process Saf. Environ., 2000, Vol. 78, 480–488.

[38] BOUROPOULOS N.CH., KOUTSOUKOS P.G., *Spontaneous precipitation of struvite from aqueous solutions*, J. Cryst. Growth, 2000, Vol. 213, 381–388.