BIODEGRADABLE CEMENT TYPE BONE IMPLANT MATERIALS BASED ON CALCIUM PHOSPHATES AND CALCIUM SULPHATE

Dominika Siek*, Joanna Czechowska, Aneta Zima, Anna Ślósarczyk

AGH University of Science and Technology,
Faculty of Materials Science and Ceramics,
30 Mickiewicza Av., 30-059 Krakow, Poland
*e-mail: siek@agh.edu.pl

Abstract

Calcium phosphates (CaPs) are widely used in hard tissue replacement because of their excellent biocompatibility. Calcium phosphate cements (CPCs) are an interesting alternative for sintered calcium phosphate ceramics due to their mouldability and self-setting properties which allow them to conform to even the most complex bone defects. However, one of the major limitations of CPCs is their relatively low resorption rate, not optimal for bone regeneration. The aim of our studies was to combine a stable hydroxyapatite with more soluble α-tricalcium phosphate (α-TCP) or calcium sulphate (CS) (resorbability: CS>>α-TCP>HA) to develop biomaterial with gradual degradation. Promising materials for use in minimally invasive surgery for bone defects repair were obtained. It was found that the degradation rate of hydroxyapatite based bone substitutes can be controlled by the addition of an appropriate kind and amount of more soluble constituent. The impact of the setting component (α-TCP or CS) on the physicochemical properties of the final products was confirmed. Furthermore the influence of organic additives (chitosan, methylcellulose, alginate) on the final materials characteristic was proven. Solutions of organic additives, applied as the liquid phases, significantly improved the workability of cement pastes. It has been demonstrated that implant materials based on calcium sulphate and α-TCP differed in their setting times, mechanical strength, dissolution rate and morphologies of apatite layers on their surfaces after soaking in simulated body fluid. The reason of observed differences is a higher susceptibility of calcium sulphate to both disintegration and degradation.

Keywords: *calcium phosphate cements, hydroxyapatite, calcium sulphate, α-tricalcium phosphate, biomaterial*

[Engineering of Biomaterials 133 (2015) 2-6]

Introduction

Nowadays calcium phosphates based bioceramics (CaPs) attracts a great interest as a material for bone substitution, mainly due to their biocompatibility and bioactivity [1-4]. CaPs sintered biomaterials may be applied in the form of powders, granules, porous and dense blocks. Calcium phosphates may also be used in the form of chemically bonded bioceramics i.e. bone cements. Calcium phosphate cements (CPCs), were invented in 1986 by Brown and Chow [5]. CPCs compose of powder and liquid phases which when mixed together create a shapeable paste. Afterward the paste is placed or injected into the bone cavity, where it sets *in situ*. Calcium phosphate cements can form hydroxyapatite (HA, $Ca_{10}(PO_4)(OH)_2$) and/or brushite ($CaHPO_4 \cdot 2H_2O$) as the reaction products. Numerous advantages such as biocompatibility, mouldability, self-setting, easy adaptation to geometrically complex shapes of defects and similarity to the bone mineral phase make CPCs an interesting alternative for sintered calcium phosphate ceramics [6,7]. Nevertheless, calcium phosphate cements have also some drawbacks which limit their clinical use. Their disadvantages include: relatively slow resorption rate, lack of macroporosity and poor mechanical properties. During the setting reaction of CPCs, nano- and micropores are formed, however the absence of interconnected macropores is believed to be a limiting factor in achieving resorption that is allied with the apposition of new bone [8-10]. A number of approaches have been applied to obtain macroporous CPCs, including additions of various soluble inorganic and organic compounds, which create the macroporosity [11-13]. Furthermore foaming agents were incorporated in the cement paste to produce synthetic calcium phosphate foams [6]. Creating macropores in the CPCs bodies by incorporation of biodegradable compounds and foaming resulted in an increase in degradation rate [14-18].

In this work a stable hydroxyapatite was combined with more soluble α-tricalcium phosphate or calcium sulphate (resorbability: CS>>α-TCP>HA) to obtain materials with gradual degradation. There are a variety of CaP cement systems and materials composed of α-tricalcium phosphate (α-TCP, $Ca_3(PO_4)_2$) are especially interesting [19]. One of the advantages of α-TCP is its ability to hydrolyze and set into calcium deficient hydroxyapatite (CDHA) at near physiological conditions from a single solid reactant. Calcium sulfate (CS) is a well-tolerated, biodegradable and osteoconductive bone graft substitute and has been proven to be effective, biocompatible component in CPCs [20,21]. Furthermore it has been reported that CS stimulates the formation of blood vessels, which has been found to be extremely important in bone formation [22,23]. The process of vascular induction is called angiogenesis, and it plays a key role in bone regeneration, because the new bone tissue formation is related to the local presence of blood vessels. Moreover, calcium sulphate based biomaterials possessed the ability to release a broad range of bioactive and therapeutic agents what makes CS an interesting component of composite-type bone cements [24]. It is known that using organic additives in the cement liquid may improve the workability of cement pastes [16].

The aim of the study was to develop and evaluate the new bone substitutes based on calcium phosphates and calcium sulphate as well as investigate the influence of powder and liquid phase composition on physicochemical properties of the final materials.

Materials and Methods

Materials

In this study four materials in the form of mouldable pastes were obtained by mixing powder (P) and liquid (L) phases in appropriate ratios. Liquid to powder ratio (L/P) was experimentally adjusted for each material. Initial powder batches were prepared by combining hydroxyapatite (HA) with α-tricalcium phosphate (α-TCP) or calcium sulphate (CS). HA and α-TCP were synthesized by the wet chemical method. CaO (POCH, Poland) and H_3PO_4 were applied to synthesize HA.

During the synthesis pH value of the slurry was stabilized at ~11 using ammonium hydroxide (POCH, Poland) solution. Hydroxyapatite precipitate was dried and calcined above 700°C (HA/c). Calcium hydroxide $Ca(OH)_2$ (MERCK, Germany) and 85% phosphoric acid (POCH, Poland) were used to obtain α-TCP. The produced α-TCP powder was sintered at 1300°C, ground in the attritor and sieved [25]. Calcium sulphate hemihydrate ($CaSO_4 \cdot 0.5H_2O$) was purchased from Acros Organics, USA. A variety of liquids, chosen during the preliminary studies (results not shown) were used in the preparation of cements. Distilled water, 1.00% chitosan solution (ALDRICH, Germany) in 0.30% CH_3COOH (POCH, Poland), 0.75% methylcellulose solution (Fluka) in 2.00% Na_2HPO_4 (CHEMPUR, Poland) and 0.75% alginate solution (Acros Organics, USA) in 2.00% Na_2HPO_4 served as liquid phases. Initial powder and liquid composition of studied cements was presented in TABLE 1.

TABLE 1. Initial composition of studied bone cements.

Cement	Powder phase (P)	Liquid phase (L)	L/P [g/g]
A	CS : HA/c 3:2	distilled water	0.54
B		chitosan solution	
C	α-TCP : HA/c 3:2	methylcellulose solution	0.50
D		alginate solution	

Specific surface area

Specific surface area of the initial powders was determined via BET (*Brunauer-Emmett-Teller*) method.

Phase composition

The phase composition (XRD, Bruker) of starting powders and the final cement bodies was checked by X-ray diffraction (XRD) using D2 PHASER (Bruker) with CuK_α radiation within the 2θ range from 10° to 90°. All crystalline phases were identified by comparison with the Joint Committee on Powder Diffraction Standards (JCPDS): HA (JCPDS 01-074-9761), α-TCP (JCPDS 00-009-0348) and calcium sulphate dihydrate (CSD) (JCPDS 00-006-0047). Phase quantification was done using the Rietveld analysis.

Setting times

The initial (I) and final (F) setting times of the cements were measured using the Gillmore needles according to the C266-08 ASTM standard [26]. Results were presented as data mean ± standard deviation.

Compressive strength

The specimens for compressive strength measurements were prepared by putting cement pastes into a cylindrical Teflon mold (6 mm in diameter and 12 mm in height). The Universal Testing Machine (Instron 3345) was used for measuring the compressive strength of specimens at the loading rate of 1 mm/min. Data were presented as mean ± standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA) and post-hoc Tukey HSD multiple comparison.

Porosity

The open porosity of hardened cement bodies was investigated via the mercury intrusion porosimetry (MIP, Auto Pore IV, Micromeritics).

***In vitro* chemical stability and bioactivity**

In order to estimate a biochemical stability of the cement measurements of pH changes and ionic conductivity in simulated body fluid (SBF) and distilled water, during the samples incubation at 37°C, were conducted. SBF was produced according to Kokubo's procedure [27]. SEM observations of the cement samples surfaces (Nova 200 NanoSEM, FEI Company) as well as analysis of the chemical composition in microareas (EDS) to evaluate the bioactive potential were done. After 7 days of incubation in SBF weight loss (WL) of specimens was calculated as follows:

$$WL(\%) = (W_0 - W_d)/W_0 \cdot 100 \quad (1)$$

where W_0 is the initial weight of the specimen and W_d is the weight of the specimen dried after incubation in SBF [28].

Results and Discussion

Specific surface area

Specific surface area of the starting powders, determined via BET method, was as follows: α-TCP - 3.81 ±0.02 m^2/g, calcium sulphate hemihydrate - 1.77 ±0.04 m^2/g and HA/c - 24.7 ±0.02 m^2/g. The obtained results may suggest the differences in reactivity of the powders during reactions accompanying setting processes.

Phase composition

The phase composition of the studied bone cements is shown in TABLE 2. The detailed analysis of the XRD patterns confirmed that calcined hydroxyapatite powder (HA/c) was monophasic hydroxyapatite. The initial α-TCP powder was composed of 94.3 ±3.5 wt.% α-TCP and 5.7 ±3.5 wt.% HA. Calcium sulphate hemihydrate and α-tricalcium phosphate were the setting components in the studied materials. In the case of material A and B higher liquid to powder ratio was established in comparison to cements C and D. Materials A and B after setting and hardening were composed of hydroxyapatite and calcium sulphate dihydrate (CSD) (TABLE 2). Presence of CSD in the final materials was the result of reaction between calcium sulphate hemihydrate and water, according to the equation (2) [29]:

$$2(CaSO_4 \cdot 0.5H_2O) + 3H_2O \rightarrow 2(CaSO_4 \cdot 2H_2O) + Q \quad (2)$$
$$(CSH) \rightarrow (CSD)$$

where Q is the amount of heat evolved.

Cements C and D consisted of two crystalline phases: α-TCP and hydroxyapatite (TABLE 2). Observed decrease in the amount of α-TCP in the set cement bodies was a result of α-TCP hydrolysis to a calcium-deficient hydroxyapatite (CDHA), according to the equation (3) [30,31]:

$$3\,\alpha\text{-}Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(HPO_4)(PO_4)_5(OH) \quad (3)$$
$$\alpha\text{-}TCP \rightarrow CDHA$$

It was concluded that about ~10-15 wt.% of α-TCP hydrolyze to CDHA in cement C and D (TABLE 2).

TABLE 2. Phase composition of studied bone cements 7 days after setting and hardening.

Cement	Crystalline phases [wt.%]		
	HA	α-TCP	CSD
A	40.2 ±2.4	-	59.8 ±2.4
B	39.1 ±2.1	-	60.9 ±2.1
C	52.9 ±1.7	47.1 ±1.7	-
D	52.2 ±1.1	47.8 ±1.1	-

Setting times

Setting process of cements based on calcium sulphate and HA/c was faster in comparison to materials consisting of α-TCP and HA/c (TABLE 3). Cements A and B set from 3 ±1 (I) to 14 ±1 (F) min, while in the case of materials C and D setting times ranged from 10 ±1 (I) to 30 ±1 (F) min. Materials based on calcium sulphate and distilled water showed the shortest setting times (I = 3 ±1, F = 6 ±1 min). Rapid setting process of material A may cause difficulties with cement preparation and application that is why it was eliminated from further studies. Introduction of the applied organic solution improved the workability of obtained cement pastes via enhancing their flexibility. On the other hand using solution of the chitosan in acetic acid significantly prolonged setting process of materials on the basis of CS and HA/c.

TABLE 3. Initial and final setting times of the cements.

Cement	Setting time [min]	
	Initial (I)	Final (F)
A	3 ±1	6 ±1
B	7 ±1	14 ±1
C	10 ±1	30 ±1
D	11 ±1	27 ±1

Compressive strength

Cements based on CS and HA/c showed comparable compressive strength (~9 MPa) (one-way ANOVA, P > 0.05). The lowest compressive strength value (2.7 ±0.6 MPa) was observed for material D consisting of α-TCP, HA/c and alginate used in the form of liquid phase (FIG. 1). The compressive strength of developed materials was comparable with cancellous bone (2-12 MPa [32]).

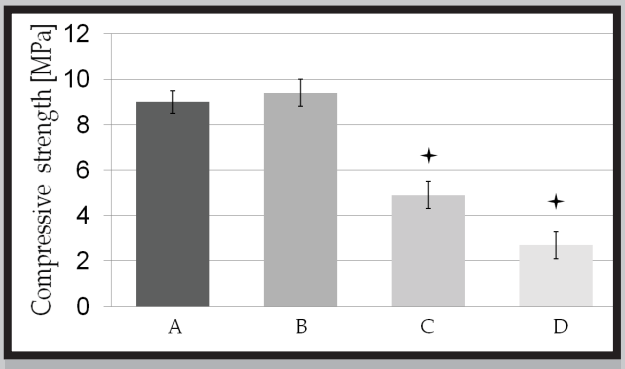

FIG. 1. Compressive strength of the cements 7 days after setting and hardening. Mean ± standard deviation, samples marked ✦ are statistically different from A and B (one-way ANOVA, p>0.05).

Porosity

Developed cements exhibited the biomodal pore size distributions with pores below 1.4 μm (A and B) and 0.5 μm (C and D) (FIG. 2). Macroporosity in CPCs is expected to be formed *in vitro* and *in vivo* via dissolution of soluble setting phases i.e. α-TCP and calcium sulphate dihydrate. Open porosity of final cement bodies was ~49 vol.% (TABLE 4).

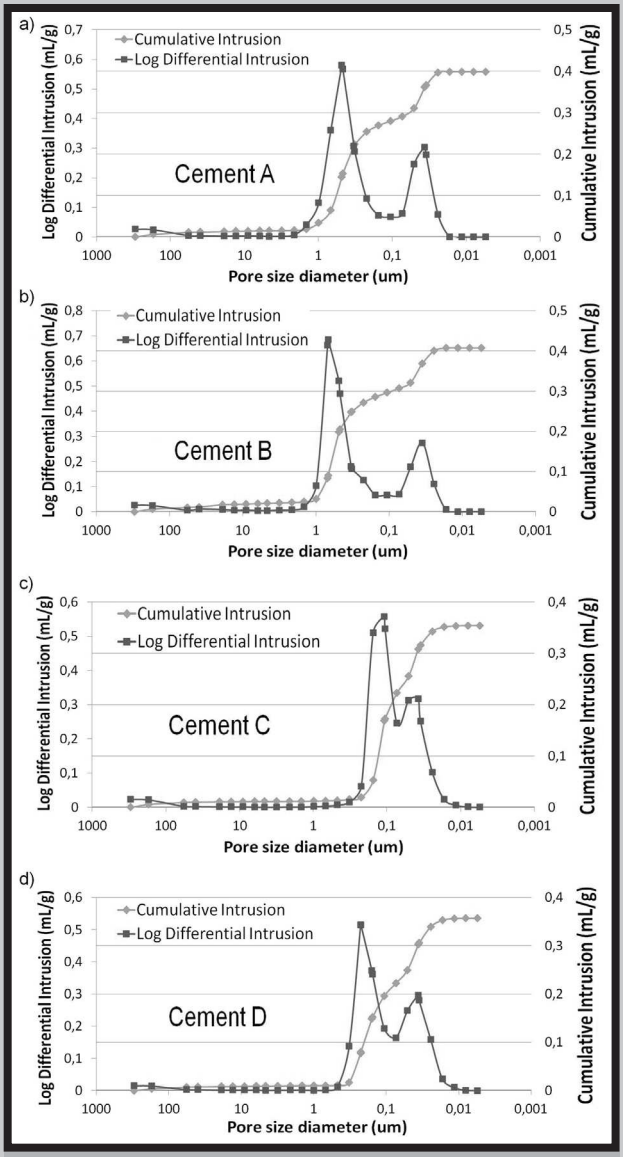

FIG. 2. Pore size distribution curves of developed cements.

TABLE 4. Open porosity and pore size distribution of the materials.

Cement	Porosity [vol. %]	Pore size distribution [μm]
A	49	0.017 – 1.400 max I: 0.040 max II: 0.480
B	49	0.017 – 1.400 max I: 0.035 max II: 0.630
C	49	0.012 – 0.320 max I: 0.043 max II: 0.120
D	49	0.012 – 0.470 max I: 0.036 max II: 0.220

Based on obtained results two materials with the most favorable physicochemical properties were chosen for further studies, namely cements B and C. Selected biomaterials exhibited not only easy molding, allowing them to conform precisely to irregular bone voids, but also optimal setting times as well as good compressive strength.

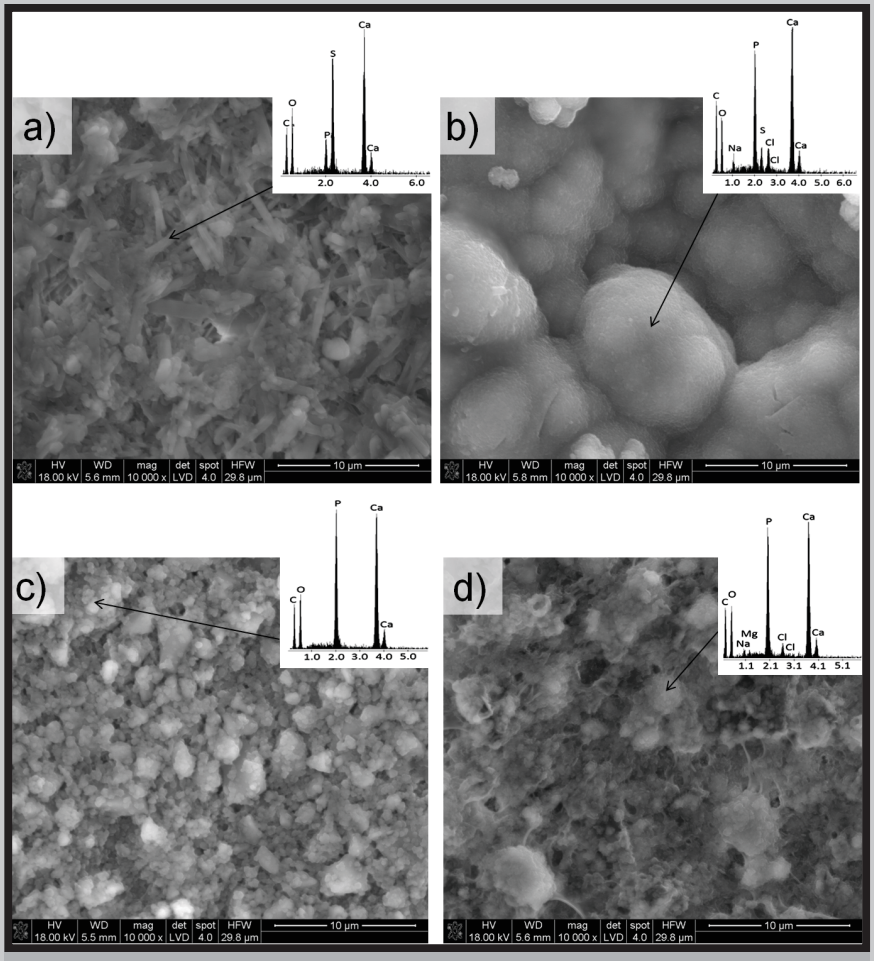

FIG. 3. SEM micrographs and chemical composition in microareas of the cement B: non-incubated (a), after 7 days of incubation in SBF (b) and cement C: non-incubated (c), after 7 days of incubation in SBF (d).

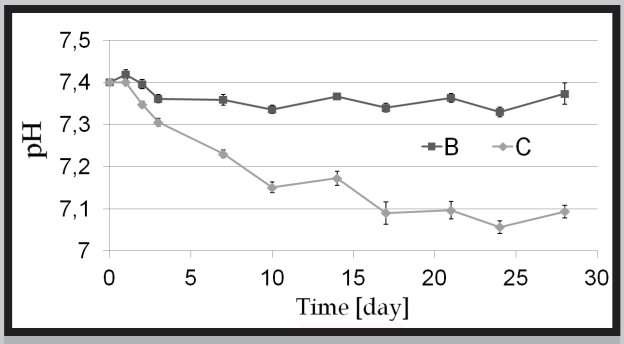

FIG. 4. pH of simulated body fluid (SBF) as a function of samples B and C incubation time.

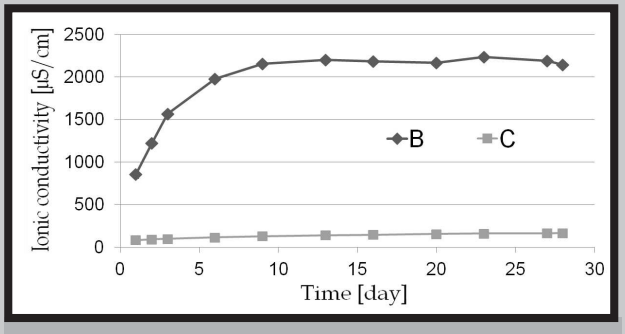

FIG. 5. Ionic conductivity vs. incubation time of samples B and C in distilled water.

In vitro chemical stability and bioactivity

In vitro studies revealed that developed materials varied in the morphologies of apatite layers on their surfaces after soaking in SBF (FIG. 3). In the case of cement B, composed of CS and HA/c with chitosan applied in the form of liquid phase, as soon as after 7 days of incubation in simulated body fluid (SBF), surface of tested material was covered with the typical cauliflower-like CaPs structures. Cement B consisted of fast resorbable calcium sulphate which is the rich source of Ca^{2+}. Released calcium ions react with phosphate ions from the SBF and create apatite layer on the surface of incubated samples. It was concluded that cement B, may be regarded as bioactive. In contrast on the surface of cement with α-TCP, HA/c and methylcellulose solution non-continuous and irregular apatite layer was observed. α-TCP dissolves slower than CSD which may be the reason for the observed differences.

In the case of both examined materials pH during 28 days of incubation in SBF was close to the physiological level (FIG. 4). Higher ionic conductivity observed for cement B was connected with CSD dissolution and thus release of high amounts of calcium and sulphate ions (FIG. 5). Implant materials based on calcium sulphate showed higher degradation rate compared to materials with α-TCP during incubation in SBF. After 7 days of soaking in SBF material B showed 20 ±1.6wt.% weight loss, while the 4 ±0.4 wt.% increase in weight for material C was observed. It confirmed different degradability and potential *in vivo* biodegradability of the studied cements.

Conclusions

New bioactive and potentially biodegradable materials based on calcined hydroxyapatite and α-tricalcium phosphate or calcium sulphate were developed. The impact of the setting component (α-TCP or CS) on the physicochemical properties of the final products was confirmed. The results of our studies demonstrated that degradation rate of CPCs can be controlled by addition of appropriate amount of more soluble component. Implant materials based on calcium sulphate and HA/c showed higher degradation rate compared to materials with α-TCP and HA/c during incubation in SBF. Obtained potential bone substitutes possessed bimodal pore size distribution and open porosity ~49 vol.%. Solutions of selected organic additives (i.e. chitosan, methylcellulose, alginate), applied as the liquid phases, significantly improved the workability of cement pastes. Cement (B) based on CS and HA/c with chitosan introduced in the form of liquid phase revealed the most favorable properties. It showed good handling, optimal initial and final setting time (I = 7 ±1 min, F = 14 ±1 min) as well as compressive strength of 9.4 ±1.0 MPa. SEM and EDS studies showed the growth of apatite layer on the surface of this material as soon as after 7 days of incubation in SBF what confirmed its bioactive character. Further biological evaluation of developed biomaterials is necessary.

Acknowledgments

The work has been supported by the Polish National Science Centre funds allocated with the decision DEC-2013/09/N/ST8/04171.

References

[1] Dorozhkin S.V., Epple M.: Biological and medical significance of calcium phosphates. Angewandte Chemie International Edition 41 (2002) 3130-3146.
[2] Polkowska I., Sobczyńska-Rak A., Szyszkowska A., Koper J., Ślósarczyk A.: Use of hydroxyapatite in the treatment of bone cavities after the removal of inflammatory lesions within the jaws - case reports. Medycyna Weterynaryjna 68[7] (2012) 436-440.
[3] Ślósarczyk A., Piekarczyk J.: Ceramic materials on the basis of hydroxyapatite and tricalcium phosphate. Ceramics International 25[6] (1999) 561-565.
[4] Wójtowicz J., Leszczyńska J., Chróścicka A., Ślósarczyk A., Paszkiewicz Z., Zima A., Rożniatowski K., Jeleń P., Lewandowska-Szumieł M.: Comparative in vitro study of calcium phosphate ceramics for their potency as scaffolds for tissue engineering, Bio-Medical Materials and Engineering 24 (2014) 1609-1623.
[5] Brown W.E., Chow L.C.: A new calcium phosphate water setting cement, In Cements Research Progress, Brown P.W., editor, American Ceramic Society: Westerville, OH, USA (1986) 352-379.
[6] Montufar E.B., Traykova T., Gil C., Harr I., Almirall A., Aguirre A., Engel E., Planell J.A., Ginebra M.P.: Foamed surfactant solution as a template for self-setting injectable hydroxyapatite scaffolds for bone regeneration. Acta Biomaterialia 6[3] (2010) 876-885.
[7] Ginebra M.P., Espanol M., Montufar E.B., Pereza R.A., Mestres G.: New processing approaches in calcium phosphate cements and their applications in regenerative medicine, Acta Biomaterialia 6[8] (2010) 2863-2873.
[8] Frankenburg E.P., Goldstein S.A., Bauer T.W., Harris S.A., Poser R.D.: Biomechanical and histological evaluation of a calcium phosphate cement. The Journal of Bone&Joint Surgery 80[8] (1998) 1112-1124.
[9] Jansen J.A., de Ruijter J.E., Schaeken H.G., van der Waerden J.P.C.M., Planell J.A., Driessens F.C.M.: Evaluation of tricalcium phosphate/hydroxyapatite cement for tooth replacement: an experimental animal study. Journal of Materials Science: Materials in Medicine 6 (1995) 653-657.
[10] Ooms E.M., Wolke J.G., van der Waerden J.P., Jansen J.A.: Trabecular bone response to injectable calcium phosphate (Ca–P) cement. Journal of biomedical materials research 61 (2002) 9-18.
[11] Takagi S., Chow L.C.: Formation of macropores in calcium phosphate cement implants. Journal of Materials Science: Materials in Medicine 12 (2001) 135-139.
[12] Xu H.H.K., Weir M.D., Burguera E.F., Fraser A.M.: Injectable and macroporous calcium phosphate cement scaffold. Biomaterials 27 (2006) 4279-4287.
[13] Ruhé P.Q., Hedberg E.L., Padron N.T., Spauwen P.H.M., Jansen J.A., Mikos A.G.: Biocompatibility and degradation of poly(dl-lactic-co-glycolic acid)/calcium phosphate cement composites. Journal of Biomedical Materials Research 74A (2005) 533-544.
[14] Khashaba R., Moussa M.M., Mettenburg D.J., Rueggeberg F.A., Chutkan N.B., Borke J.L.: Polymeric-Calcium Phosphate Cement Composites-Material Properties: In Vitro and In Vivo Investigations. International Journal of Biomaterials (2010).
[15] Moreau J., Xu H.H.K.: Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate - chitosan composite scaffold. Biomaterials 30[14] (2009) 2675-2682.
[16] Pere R.A.,, Kim H.W., Ginebra M.P.: Polymeric additives to enhance the functional properties of calcium phosphate, Journal of Tissue Engineering 3[1] (2012) 2041731412439555.
[17] Wang X., Ma J., Wang Y., He B.: Structural characterization of phosphorylated chitosan and their applications as effective additives of calcium phosphate cements. Biomaterials 22[16] (2001) 2247-2255.
[18] Zhou W., Xue Y., Ji X., Yin G., Zhang N., Ren Y.: A novel injectable and degradable calcium phosphate/calcium sulfate bone cement. African Journal of Biotechnology 10[88] (2011) 19449-19457.
[19] Czechowska J., Zima A., Paszkiewicz Z., Lis J., Ślósarczyk A.: Physicochemical properties and biomimetic behaviour of α-TCP-chitosan based materials. Ceramics International 40 (2014) 5523-5532.
[20] Zima A., Paszkiewicz Z., Siek D., Czechowska J., Ślósarczyk A.: Study on the new bone cement based on calcium sulfate and Mg, CO_3 doped hydroxyapatite. Ceramics International 38[6] (2012) 4935-4942.
[21] Olkowski R., Kaszczewski P., Czechowska J., Siek D., Pijocha D., Zima A., Ślósarczyk A., Lewandowska-Szumieł M.: Cytocompatibility of the selected calcium phosphate based bone cements: comparative study in human cell culture. Journal of Materials Science: Materials in Medicine. 26[12] (2015) 270.
[22] McNeill S.R., Cobb C.M., Rapley J.W., Glaros A.G., Spencer P.: In vivo comparison of synthetic osseous graft materials: a preliminary study. Journal of Clinical Periodontology 26 (1999) 239-245.
[23] Strocchi R., Orsini G., Iezzi G., Scarano A., Rubini C., Pecora G., Piattelli A.: Bone regeneration with calcium sulfate: evidence for increased angiogenesis in rabbits. Journal of Oral Implantology 28[6] (2002) 273-278.
[24] Orellana BR, Hilt J.Z., Puleo D.A.: Drug release from calcium sulfate-based composites. Journal of Biomedical Materials Research Part B: Applied Biomaterials 103[1] (2014) 135-142.
[25] Ślósarczyk A., Paszkiewicz Z., Sposób wytwarzania wysokoreaktywnych proszków fosforanów wapnia, (2005) PL 190 486
[26] ASTM C266-04: Standard test method for time setting of hydraulic-cement paste by gillmore needles, ASTM Annual Book of standards, PA 19428-2959, USA.
[27] Takadama H., Kokubo T.: In vitro evaluation of bioactivity, In: Bioceramics and their clinical applications, Kokubo T., editor, Cambridge UK: Woodhead Publishing (2008) 165-183.
[28] Xue Z., Zhang H., Jin A., Ye J., Ren L., Ao J., Feng W., Lan X.: Correlation between degradation and compressive strength of an injectable macroporous calcium phosphate cement. Journal of Alloys and Compounds 520 (2012) 220-225.
[29] Singh N.B., Middendorf B.: Calcium sulphate hemihydrate hydration leading to gypsum crystallization. Progress in Crystal Growth and Characterization of Materials 53[1] (2007) 57-77.
[30] Camiré C.L., Gbureck U., Hirsiger W., Bohner M.: Correlating crystallinity and reactivity in an α-tricalcium phosphate. Biomaterials 26[16] (2005) 2787-2794.
[31] Durucan C., Brown P.W.: α-Tricalcium phosphate hydrolysis to hydroxyapatite at and near physiological temperature. Journal of Materials Science: Materials in Medicine 11[6] (2000) 365-371.
[32] Bartolo PJ.: Virtual and Rapid Manufacturing: Advanced Research in Virtual and Rapid, Taylor&Francis Group, London (2007) 64.