New bone implant material with calcium sulfate and Ti modified hydroxyapatite

A. Ślósarczyk*, J. Czechowska, Z. Paszkiewicz, A. Zima
AGH University of Science and Technology,
Al. Mickiewicza 30, 30-059 Kraków, Poland
* Corresponding author: E-mail address: aslosar@agh.edu.pl

Materials

ABSTRACT

Purpose: In this work, calcium sulfate hemihydrate (CSH) was combined with titanium doped hydroxyapatite (TiHA) to develop a novel bone cement. Results of previous studies showed that bioactive potential of titanium modified hydroxyapatite ceramics is higher than that of pure HA. Calcium sulfate hemihydrate is also considered as a safe, biocompatible material, however it has been criticized for its rapid resorption. Combination of these materials may result in new cement type material with surgical handiness and selective resorption.

Design/methodology/approach: TiHA was obtained by a wet method. Three compositions with different CSH:TiHA weight ratios, namely 3:2, 2:3 and 1:4 were examined. Pure CSH was used as a reference. Distilled water and Na_2HPO_4 solutions were applied as liquid phases. The study presents the setting time (Gillmore apparatus), phase composition (XRD), microstructure (SEM), porosity (mercury porosimetry) and compressive strength of the obtained new, cement type, implant material.

Findings: Initial (I) and final (F) setting times of the obtained cements differed in the range of 2-16 min (I) and 4-75 min (F). The phase composition of the hardened cement bodies characterized by XRD method revealed the presence of calcium sulfate dihydrate (CSD) and hydroxyapatite. Scanning electron microscopy images show excellent bonding between needle-like CSD crystals and apatitic phase. Porosity of the final samples varied from 49 to 59% with pore size diameter from 5 nm to 3.0 μm. Compressive strength of the samples differed in the range of 3.81-7.58 MPa.

Research limitations/implications: The obtained results suggest that CSH-TiHA cements have the potential to be applied in bone substitution and for delivery of drugs. Bioactivity and biodegradation of the studied materials should be checked.

Originality/value: According to our knowledge, these are the first studies concerning surgical handiness of bone implant materials based on calcium sufate hemihydrate and titanium doped hydroxyapatite. The cement type composites are biocompatible, shapeable and easy to apply and adapt in bone defects.

Keywords: Bone substitute; Titanium modified hydroxyapatite; Calcium sulfate

1. Introduction

Autografts are still considered as a gold standard of bone grafting. However, autogenous cancellous bone is of limited availability and is also accompanied by high morbidity and mortality during the harvest. Furthermore, this method has restricted application in the case of bigger bone defects [1]. Many problems are also associated with allogenous bone grafts [2]. In the light of the above limitations, development of efficient, bioactive artificial bone substitutes has been promoted. Nearly 30 years ago LeGeross [3] as well as Brown and Chow [4] first evaluated calcium phosphate cements (CPCs) for medical applications. CPCs bone cements are obtained by mixing one or several calcium phosphate powders with an aqueous solutions to produce a paste that hardens within a restricted period of time. The injectable materials are also available [5,6]. The final cement properties depend on the characteristics of solid and liquid phases, liquid to powder ratio and reaction conditions. A large number of calcium phosphate cements, thanks to their self-setting ability, excellent biocompatibility and capability of being replaced by new bone have found wide clinical applications for repair of bone defects and bone augmentation [7,8]. Calcium phosphate-based bioceramics, including hydroxyapatite (HA) and doped HA are very popular in use as bone substitutes [9-12]. Results of previous studies showed that bioactive potential of titanium modified hydroxyapatite ceramics is higher than that of pure HA which makes this material very interesting from the medical point of view [13].

Calcium sulfate (CS) occupies a unique position in the group of regenerative materials. Calcium sulfate hemihydrate (CSH) and calcium sulfate dihydrate (CSD) are widely recognized as safe and bioactive implant materials and have been already successfully used in bone substitution [1,14-19]. Despite these advantages, calcium sulfate has not reached the popularity of many other regenerative materials and has been criticized for its rapid resorption. The resorption rate of CSD is considered to be too fast to manage gradual replacement of biomaterial by the newly formed bone. However, for the same reason, calcium sulfate found a new application and started to be used as a fast resorbable bone cements' component to produce porosity in vivo [8,18]. The hemihydrate form of CS reacts with water and creates a resorbable phase consisting of calcium sulfate dihydrate.

The reaction results in the growth of interlocking needle–like crystals which form the set cement. Besides serving as a bone filler, calcium sulfate has also been used as a drug delivery material [1,12].

The aim of this study was to obtain a novel bone cement with surgical handiness and selective resorption based on titanium modified hydroxyapatite and calcium sulfate. Physico-chemical properties of the new bone implant material were evaluated.

2. Materials and methods

The investigated self-setting cements were produced using titanium doped hydroxyapatite (TiHA) and calcium sulfate hemihydrate (CSH). Hydroxyapatite doped with 2.0 wt % of titanium was synthesized by a wet method using chemical grade reactants: CaO (POCH, Poland), 85% solution of H_3PO_4 (POCH, Poland) and 15% solution of $TiCl_3$ in 10% HCl (MERCK,

Germany). The obtained Ti modified HA powder was ground to the grain size below 0.06 mm and sieved. Calcium sulfate hemihydrate - CSH (Acros Organics) was used as a second component. Three different powder batches were prepared by mixing the CSH with TiHA at different weight ratios, namely: TiHA /CS_60 - 3:2, TiHA/CS_40 - 2:3 and TiHA/CS_20 - 1:4. Mixing was performed using a mixer mill (Retsch MM400, 5Hz/5min). Pure CSH served as a reference. Distilled water and Na_2HPO_4 solutions (1.25% and 2.5%) were applied as liquid phases.

2.1. Setting times

Initial (I) and final (F) setting times of the varied formulations were determined using Gillmore apparatus according to ASTM C266-04 specification [20]. The self–setting cement samples were prepared by mixing powder batches with liquid phases, according to experimental protocol (at the given L/P ratios for achieving appropriate rheological properties of the pastes). All experiments were performed at 23±2°C. Each measurement was repeated 10 times and the average value was calculated.

2.2. Phase composition

Phase composition of the hardened cement bodies was characterized by X-ray diffraction method (Philips diffractometer, X'Pert Pro). Phase quantification was made according to the Rietveld method.

2.3. Microstructure

Cross-section microstructure of fractured set cements was examined by scanning electron microscopy (SEM, Nova NanoSem 200). All samples were previously sputtered with carbon to avoid charging effect. Chemical compositions in microareas were determined by EDS (Model 4457F_ISUS-SN).

2.4. Porosity

Open porosity and pore size distribution of the samples were evaluated using mercury intrusion porosimetry (MIP) (AutoPore Porisimeter, Micromeritics). The dried samples were introduced into the penetrometer and placed in the low-pressure chamber of the apparatus, where they were deaerated. Mercury was subsequently introduced into the penetrometer and the penetration volume was registered while increasing the intrusion pressure. After low-pressure measurements, the penetrometer was removed and placed inside the high-pressure chamber where the high pressure measurements were carried out.

2.5. Compressive strength

Compressive strength of the materials was assessed. Samples for compression tests were obtained by putting cement pastes into

teflon-moulds (6 mm in diameter and 12 mm in height) where they were allowed to set. Specimens were removed from the moulds after the final setting time and then stored at 37°C, in humid environment. After 1, 7, 14 and 28 days samples were tested in compression using an universal testing machine (Instron 3345) with cross-head speed of 2 mm/min, until failure. Dimensions of the samples were measured with accuracy of 0.01 mm using a micrometer. The average compressive strength was calculated from at least 10 measurements for each time point.

3. Results

3.1. Setting times

The cement setting times depended on the amount of CSH and TiHA, concentration and kind of mixing liquid. Setting times of the studied materials are presented in Table 1. Calcium sulfate hemihydrate was able to set at a lower L/P ratio, however the experiment was performed at a fixed L/P ratio (L/P=0.52) for better illustration of the influence of the TiHA amount in the cement composition on the setting time. Liquid to powder ratios for TiHA/CS_60, TiHA/CS_40, TiHA/CS_20 were established as 0.54, 0.58, 0.64 ml/g respectively. Consistency of the obtained moldable pastes enable their easy application to the bone defect. Lower amount of TiHA in composition resulted in decreasing required L/P ratio as well as in prolonged setting time (Fig. 1).

When the amount of TiHA exceeds 80 wt%, too long setting time of cement makes it unacceptable as a bone filler. Initial (I) and final (F) setting times of the cements differed in the range of 2-16 min (I) and 4-75 min (F). The shortest setting times were achieved when distilled water was used as the liquid phase. Na_2HPO_4 acted as a setting time retarder and influenced mainly the initial setting time. On the other hand, it changed the interval between initial and final setting times only slightly (Table 1).

The main reaction that takes place during the process of cement setting is the formation of calcium sulfate dihydrate, eq.(1).

$$2\ CaSO_4 \cdot 0.5H_2O + 3\ H_2O \rightarrow 2\ CaSO_4 \cdot 2H_2O \qquad (1)$$
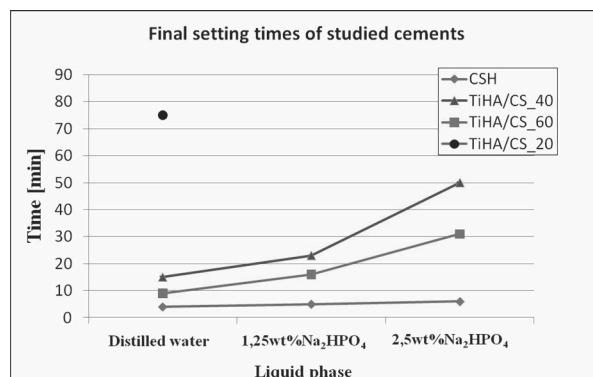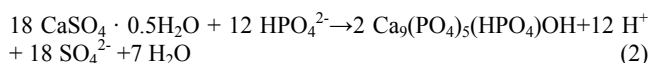

Fig. 1. Dependence of the final setting time on the applied liquid phase

However, when CSH and phosphate ions are simultaneously present in the cement paste, calcium ions are able to precipitate with phosphate ions according to eq.(2).

$$18\ CaSO_4 \cdot 0.5H_2O + 12\ HPO_4^{2-} \rightarrow 2\ Ca_9(PO_4)_5(HPO_4)OH + 12\ H^+ + 18\ SO_4^{2-} + 7\ H_2O \qquad (2)$$

During the reaction, hydronium ions are released which reduces local pH values and may promote the increased solubility of TiHA particles in the cement paste. The influence of phosphate ions on the setting time has been already observed by Bohner [18]. His experiments revealed that in the cement material consisting of α-TCP and CSD the presence of phosphate ions decreases setting time by increasing α-TCP solubility and hence its transformation to CDHA. In our study, the only setting agent was CSH and the reverse effect was noticed. Phosphate ions affected the initial setting time by disturbing the setting reaction of calcium sulfate hemihydrate to CSD, which was experimentally observed.

3.2. Phase composition

XRD analysis after 7 days showed that all cements were composed of hydroxyapatite and calcium sulfate dihydrate (Fig. 2).

Table 1.
Setting times of the studied cement compositions

Liquid phase	Material	L/P	Initial setting time I [min]	Final setting time F [min]
Distilled water	CSH	0.52	2	4
	TiHA /CS-60	0.54	4	9
	TiHA /CS-40	0.58	8	15
	TiHA /CS-20	0.64	15	>75
1.25 wt% Na_2HPO_4	CSH	0.52	4	5
	TiHA /CS-60	0.54	11	16
	TiHA /CS-40	0.58	15	23
2.5 wt% Na_2HPO_4	CSH	0.52	5	6
	TiHA/CS-60	0.54	25	31
	TiHA/CS-40	0.58	27	50

A. Ślósarczyk, J. Czechowska, Z. Paszkiewicz, A. Zima

Results of XRD analysis, summarized in Table 2, prove that the whole calcium sulfate hemihydrate reacted with the aqueous solutions and created calcium sulfate dihydrate so the hydratation reaction was completed. The content of CSD phase in final products ranged from 24 to 67 wt%.

3.3. Microstructure

SEM investigations revealed that the composition of initial cement mixtures had a noticeable influence on the hardened body microstructures. A gradual change of microstructure from typical of CSH to the cement containing 80 wt% of TiHA was observed. After mixing with water, calcium sulfate hemihydrate quickly set to form a calcium sulfate dihydrate crystal matrix (Fig. 4a). The needle-shaped crystals with smooth surface formed an interconnected network. Only a small amount of coexisting plate-like CSD crystals were detected. The sizes of CSD grains varied from about 5 to 10 μm. The TiHA particles present in the hardened cement bodies formed aggregates located between the CSD crystals and attached to them. Inside this matrix, empty cavities, 1.5-3.0 μm in size, could be occasionally observed. Voids between CSD crystals were filled in most cases by apatitic grains and they both created microstructure of the bi-phasic material. Cements with higher amount of calcium sulfate revealed better entanglement of CSD crystals and better bonding with TiHA grains (Figs. 3b-d).

The highest porosity was obtained for the cement containing 20 wt% of CSD (TiHA/CS_20) which can suggest that this material will exhibit the lowest mechanical strength. Microphotographs of all cements show also groups of clustered precipitated apatitic forms on the surface of calcium sulfate dihydrate crystals (Fig. 4).
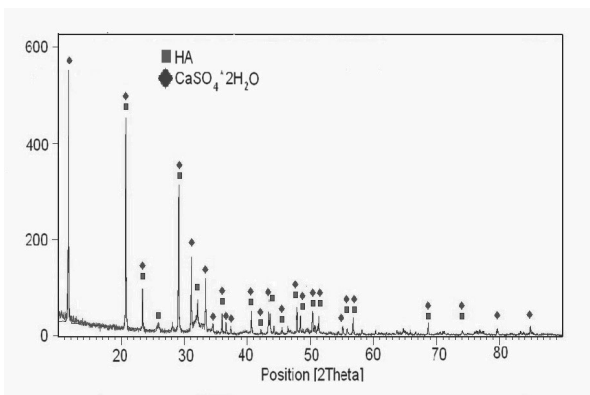

Fig. 2. Post-setting XRD pattern of the TiHA/CS_60 cement

Table 2.
Phase composition of the studied cements

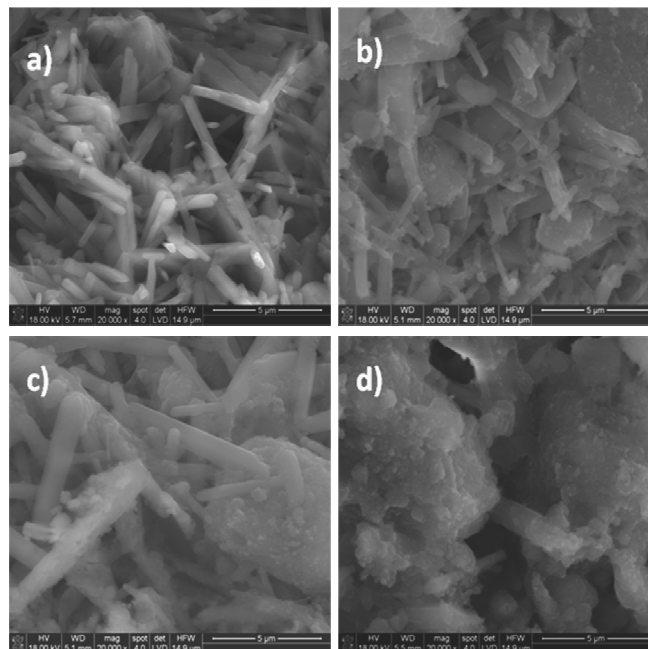| Cement	HA [wt%]	CSD [wt%]
TiHA/CS-60	33	67
TiHA/CS-40	52	48
TiHA/CS-20	76	24

Fig. 3. SEM images of fractured hardened cement bodies: a) CSD, b) TiHA/CS_60, c) TiHA/CS_40,d) TiHA/CS_20

3.4. Porosity

Mercury porosimetry analysis of the set cements showed a bimodal pore size distribution (Fig. 5). In all cements the pore size ranged from 5 nm to 3 μm. Three kinds of pores were present in the examined materials connected with: voids between CSD crystals, pores inside TiHA aggregates and voids between TiHA aggregates. The two peaks of MIP curves illustrate the existence of clearly marked populations of pores in two ranges (range I: 0.005-0.13 μm, range II: 0.06-3 μm). In the case of CSD material only one peak centered between 60 nm and 3 μm appeared at a MIP curve (Fig.5a). This peak correlates well with bigger pores in the TiHA-CS composites. These pores correspond to the voids created between the entanglements of needle-like CSD crystals, whereas the first peak (0.005-0.13 μm) corresponded to cavities formed between TiHA grains aggregates. Shape of this peak indicates a population of pores dispersed over a wide size range. In the case of TiHA/CS_20 material an additional peak (max. 60 nm) was observed confirming the presence of larger cavities between TiHA agglomerates. Increasing amount of TiHA, whose grains tended to form aggregates, impeded creation of dense interlocked needle-like CSD crystal structure. Simultaneously, it introduced an additional group of pores associated with voids between TiHA aggregates. These two effects lead to greater open porosity of a cement material. These results are in good agreement with SEM observations.

Mercury porosimetry method assumes that pores are geometrically regular and interconnected. In cement-based materials large internal pores are accessible by very narrow throats. The MIP technique determines the size of these pores as having the diameter of their throats. This phenomenon is known as the "ink bottle" effect [21]. Mercury will enter and fill the pore cavity at the pressure that is determinate by entrance opening and during the depressurization will stay entrapped inside the wider inner pore. No mismatch between calcium sulfate dihydrate intrusion and extrusion curves was observed in the range of 5 nm to 0.13 μm. Hysteresis between intrusion and extrusion curves of TiHA- CSH cements indicates the existence of ink-bottle pores in our materials (Figs. 5g-h). The observed hysteresis effect is very small but the presence of ink-bottle pores can indicate that in the studied materials larger group of bigger pores than those detected by MIP method may exist.

3.5. Compressive strength

The setting time of commercial CPCs cements is usually close to 10 min, but the setting reaction is longer - typically several days [16]. During the setting, compressive strength of cement paste usually increases to the value established after the end of setting reaction. In our studies, the increase in compressive strength during the incubation at 37 °C (humid environment) up to 14 days was observed for the material containing 60 wt% of CSD. The biggest change of compressive strength for this material was observed 7 days after setting. Compressive strength of TiHA/CS_40 remained unchanged in time (Fig. 6).
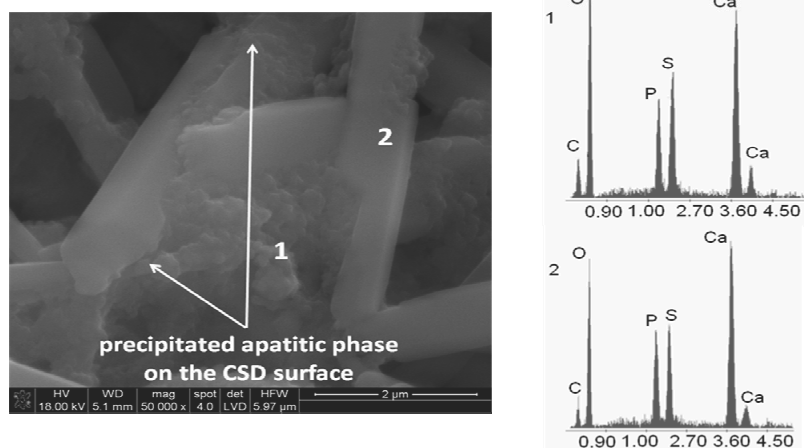

Fig. 4. EDX analysis of the crystals on the fractured surface of the TiHA/CS_40 cement sample. The arrows indicate precipitated apatitic phase present on the CSD crystal surface

Table 3.
MIP results of the pore size distribution and open porosity

Cement material	Open porosity[%]	Pore size diameter [μm]	
		range I	range II
CSD	37	-	3.0-0.06 (peak max. 0.55)
TiHA /CS_60	49	0.07-0.005 (peak max. 0.017)	3.0-0.07 (peak max. 0.40)
TiHA /CS_40	53	0.12-0.006 (peak max. 0.017)	2.5-0.12 (peak max. 0.53)
TiHA /CS_20	59	0.13-0.006 (peak max. 0.015)	2.5-0.13 (peak max. 0.83)

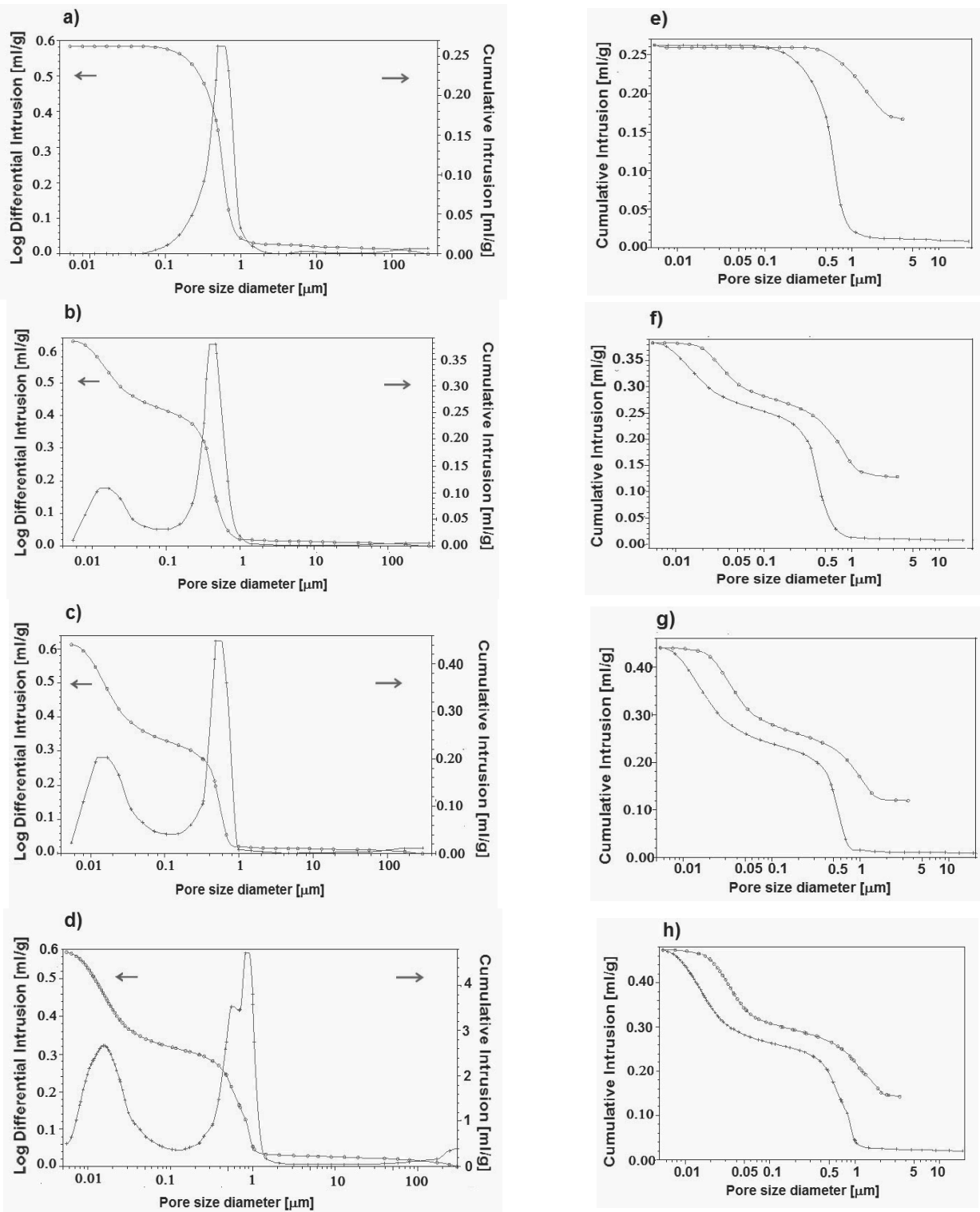A. Ślósarczyk, J. Czechowska, Z. Paszkiewicz, A. Zima

Fig. 5. Pore size distribution curves of the examined set materials: a) CSH, b) TiHA/CS_60, c) TiHA/CS_40 d) TiHA/CS_20. Curves representing intrusion (red curve) and extrusion (green curve) processes for the studied cements: e) CSH, f) TiHA/CS_60, g) TiHA/CS_40, h) TiHA/CS_20

During 28 days no significant difference in compressive strength of the examined material was observed. The amount of calcium sulfate in the initial cement powder composition influenced mechanical properties of the final samples. Increasing amount of calcium sulfate resulted in higher compressive strength. The maximum compressive strength was equal to 7.58±1.45 MPa for TiHA/CS_60 and 3.90±1.09MPa for TiHA/CS_40.

4. Conclusions

The new bone substitute based on titanium modified hydroxyapatite (TiHA) and calcium sulfate hemihydrate (CSH) with selective resorption rate was developed. A biphasic, mouldable bone cements have been prepared using TiHA and CSH powder components and water or Na_2HPO_4 aqueous solutions as liquid phases. Ater mixing CSH reacts with water creating calcium sulfate dihydrate (CSD) which acts as a binder for TiHA particles.

The cements set within 4-75 min depending on the initial powder composition and the kind of liquid phase applied. Presence of phosphate ions from Na_2HPO_4 retarded the setting process. However, when evaluating suitability of the setting time for surgical practice the use of distilled water or 1.25 wt% and lower concentration Na_2HPO_4 solutions should be considered.
The amount of CSH incorporated to the cement mixture has a significant influence on the setting time, final microstructure and compressive strength of hardened cement bodies. The minimum content of CSH phase in initial powder compositions was estimated to be 40 wt%. Cements with lower amount of CSH are not useful for implantology due to their too long setting times. The CSH:TiHA weight ratio equal to 3:2 (TiHA/CS_60) seems to be optimal to achieve cements with the appropriate properties for easy application to the bone defect.

Complex microstructure of the investigated materials was observed. Grains of TiHA were embedded in the CSD matrix composed mainly of needle-like and a small amount of plate-like crystals. Presence of apatitic phase on the surface of CSD crystals was confirmed. Attachment of TiHA grains to CSD needle-like crystals and filling the pores with hydroxyapatite particles can lead to the cement with dense interconnected microstructure that can show good mechanical properties immediately after the implantation. After implantation, CSD will be resorbed and replaced by the newly formed bone tissue.

Mercury porosimetry method can be successfully applied to describe the porous microstructure of the obtained materials. MIP investigations confirmed the influence of cement composition on the porosity and the pore size distribution. Decreasing amount of CSH in the initial powder composition causes the increase in open porosity from 49% to 59% and decrease in compressive strength. The examined cements exhibit bimodal pore size distribution in the range of 5 nm to 3 μm.

The obtained results suggest that TiHA-CSH cements reveal good surgical handiness and have the potential for applications in bone substitution and for delivery of drugs. Bioactivity and other mechanical properties of the evaluated materials should be checked. Further research is going on.
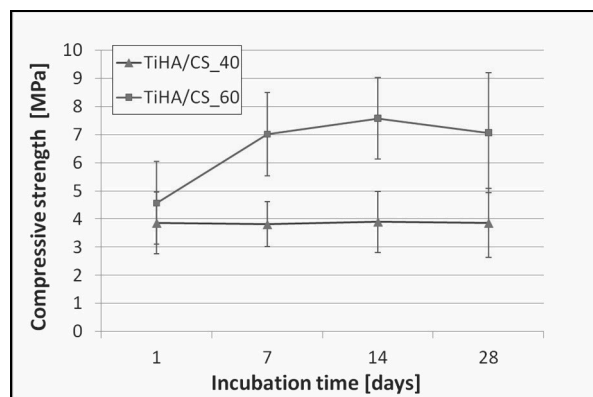

Fig. 6. Compressive strength of the examined cement compositions as a function of time

Acknowledgements

References

[1] T. Kokubo, Bioceramics and their clinical applications, Woodhead Publishing Limited, Cambridge England 14 (2008) 302-325.

[2] W. Schlickewei, C. Schlickewei, The use of bone substitutes in the treatment of bone defects, Biomaterials in Regenerative Medicine, Proceedings of the International Conference, Vienna, October 22-25, 2006, ed. A.J Nadolny.

[3] RZ. LeGeros , A. Chohayeb, A. Shulman, Apatitic calcium phosphates: possible dental restorative material, Journal of Dental Research 61 (1982) 343-347.

[4] WE. Brown, LC. Chow, A new calcium phosphate setting cement, Journal of Dental Research 62 (1983) 672-679.

[5] M. Bohner, G. Baroud, Injectability of calcium phosphate pastes, Biomaterials 26 (2005) 1553-1563.

[6] M. Nilsson, L. Wielanek, J.S. Wang, K.E. Tanner, L. Lingren, Factors influencing the compressive strength of an injectable calcium sulfate-hydroxyapatite cement, Journal of Materials Science: Materials in Medicine 14 (2003) 399-404.

[7] S. Del Valle, N. Mino, F. Munoz, A. Gonzalez, JA. Planell, MP. Ginebra, In vivo evaluation of an injectable Macroporous Calcium Phosphate Cement, Journal of Materials Science: Materials in Medicine 18 (2007) 353-361.

[8] Gangfeng Hu, Luwei Xiao, Hong Fu, Dawei Bi, Haitao Ma, Peijian Tong, Study on injectable and degradable cement of calcium sulphate and calcium phosphate for bone repair, Journal of Materials Science: Materials in Medicine 21 (2010) 627–634.

[9] C. Paluszkiewicz, A. Ślósarczyk, D. Pijocha, M. Sitarz, M. Bućko, A. Zima, A. Chróścicka, M. Lewandowska-Szumieł, Synthesis, structural properties and thermal stability of Mn-doped hydroxyapatite, Journal of Molecular Structure 976 (2010) 301-309.

[10] A.Ślósarczyk, M. Potoczek, Z. Paszkiewicz, A. Zima, M. Lewandowska-Szumieł, A. Chróścicka, Fabrication, characteristics and biological evaluation of highly porous hydroxyapatite bioceramics, Ceramic Materials 62 (2010) 224-229.

[11] M. Nilsson, L. Wielanek, J.S. Wang, K.E. Tanner, L. Lingren, Factors influencing the compressive strength of an injectable calcium sulfate-hydroxyapatite cement, Journal of Materials Science: Materials in Medicine14 (2003) 399-404.

[12] M.A. Rauschmann, T.A. Wichelhaus, V. Stirnal, E. Dingeldein, L. Zichner, R. Schnettler, V. Alt, Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier material for local delivery of antibiotics in bone infections, Biomaterials 26 (2005) 2677-2684.

[13] A. Ślósarczyk, A. Zima, Z. Paszkiewicz, J. Szczepaniak, A.H. De Aza, A. Chroscicka, The Influence of Titanium on Physicochemical Properties of Ti-modified Hydroxyapatite Materials, Proceedings of the 11th ECERS Conference, Krakow, 2009.

[14] M. Adwent, M. Cieslik, Z. Jaegermann, J. Skowronek, M. Duda, R. Orlicki, D. Sabat, T. Cieslik, Healing of Rabbit Mandibale Bone Defect filled with hemihydrate Calcium Sulfate, Polish Journal of Environmental Studies 18 (2009) 84-87.

[15] A. Lazary, B. Balla, J. P. Kosa, K. Bacsi, Z. Nagy, I. Takacs, P. P. Varga, G. Speer, P. Lakatos, Effect of gypsum on proliferation and differentiation of MC3T3-E1 mouse osteoblastic cells, Biomaterials 28 (2007) 393-399.

[16] Peng Wang, Eun-Jung Lee, Chee-Sung Park, Byung-Ho Yoon, Du-Sik Shin, and Hyoun-Ee Kim, Calcium Sulfate Hemihydrate Powders with a Controlled Morphology, The Journal of the American Ceramic Society 91 (2008) 2039-2042.

[17] M. Nilsson, E. Fernandez, S. Sarda,L. Lidgren, JA. Planell, Characterization of a novel calcium phosphate/sulphate bone cement, Journal of Biomedical Materials Research 61 (2002) 600-607.

[18] M. Bohner, New hydraulic cements based on α-tricalcium phosphate–calcium sulfate dihydrate mixtures, Biomaterials 25 (2004) 741-749.

[19] E. Fernandez, MD. Vlad, M. Montserrat Gel, J. Lopez, R. Torres, JV. Cauich, M. Bohner, Modulation of porosity in apatitic cements by the use of α-tricalcium phosphate-calcium sulphate dihydrate mixtures, Biomaterials 26 (2005) 3395-3404.

[20] ASTM C266-04. ASTM Annual Book of standards, Standard Test Method for Time Setting of Hydraulic-Cement paste by Gillmore Needles, West Conshohocken, PA 19428-2959,USA.

[21] A.B. Abell, K.L. Willis and D.A. Lange, Mercury Intrusion Porosimetry and Image Analysis of Cement-Based Materials, Journal of Colloid and Interface Science 211 (1999) 39-44.