Archives of Materials Science and Engineering

Volume 54
Issue 2
April 2012
Pages 53-59

International Scientific Journal
published monthly by the
World Academy of Materials
and Manufacturing Engineering

Influence of liquid phase on physical properties of the new triphasic bone cement

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

Received 12.02.2012; published in revised form 01.04.2012

ABSTRACT

Purpose: The aim of this work was to develop a new bone cement based on hydroxyapatite (HAp), βTCP and calcium sulfate hemihydrate (CSH) and to determine the influence of a liquid phase, used for cement pastes preparation, on physical properties of the final implant material.

Design/methodology/approach: The powder phase consisting of CSH (60 wt.%) and HAp+ βTCP (40 wt.%) was applied. Composite samples were prepared using distilled water, chitosan and methylcellulose solutions as the liquid phases. Rheological properties of the solutions were measured by Brookfield rheometer. Initial and final setting times of the cement pastes were determined. Phase composition of hardened bodies was established using XRD method. Microstructure was investigated by SEM while pore size distribution by mercury porosimetry. Compressive strength was measured by Instron Universal Testing Machine.

Findings: According to the conducted rheological measurements of the methylcellulose and chitosan solutions as well as evaluated cement pastes and hardened bodies properties, the optimal setting liquids were chosen.

Research limitations/implications: The evaluation of a biological response to the developed materials, including in vitro and in vivo experiments, need to be done.

Practical implications: The possibility of creation the physical properties of setting in vivo composites, designed for filling bone defects, via establishing the suitable liquid phase was confirmed.

Originality/value: The new composite type triphasic bone substitute, based on CSH, HAp and βTCP, with superior resorbability in comparison to the commercially available calcium phosphate bone cements was developed. The influence of liquid phase on the microstructure and mechanical strength of this implant material was determined.

Keywords: Bone substitute; Calcium phosphate; Calcium sulfate; Cement

Reference to this paper should be given in the following way:

A. Ślósarczyk, N. Osypanka, J. Czechowska, Z. Paszkiewicz, A. Zima , Influence of liquid phase on physical properties of the new triphasic bone cement, Archives of Materials Science and Engineering 54/2 (2012) 53-59.

MATERIALS

1. Introduction

Calcium phosphate cements (CPCs) are obtained by mixing appropriate amounts of powder phase, consisting of calcium phosphates and liquid phase, to create a mouldable paste. After introduction into the defect the paste fills it, sets and hardens in situ as a result of precipitation process of one or more calcium phosphates [1-3]. Nowadays calcium phosphate cements are widely used in cranioplasty, vertebroplasty, maxillofacial surgery and as drug carriers (in the drug delivery) [4-6]. Many discoveries and developments in the field of CPCs have been done during the last thirty years since 1983, when Brown and Chow obtained the first calcium-phosphate cement [1]. Despite the large number of information collected in the past 2 decades, the ideal bone cement still does not exist. There are a number of requirements that bone implant material needs to match, including biocompatibility, bioresorbability, surgical handiness, sufficient mechanical properties providing support for new forming tissues and low costs. Ideally, appropriate mechanical properties should be reached within couple of minutes after initial setting [7]. Cement pastes should set slowly enough to provide sufficient time to perform implantation but on the other hand fast enough to prevent launching the operation. Some CPCs can be injected directly into fractures due to their superior rheological properties [8]. Furthermore, the choice of constituents and development of bioresorbable materials with ability to undergo a progressive degradation while new tissue is created have become a very important issue. Hydroxyapatite has been found to be the most stable phase among the calcium phosphates therefore the implants made of calcined HA can be noticed in bone defects many years after implantation. For this reason, biomaterials composed of more soluble calcium orthophosphates, such as β-TCP, α-TCP and/or biphasic calcium phosphate HA+TCP (BCP) are preferable for biomedical applications [9-13]. Furthermore, experimental results showed that BCP had a higher ability to adsorb fibrinogen, insulin or type I collagen than HA [14]. Calcium sulfate (CS) possesses a long history of clinical use and is known to be a well-tolerated, rapidly and completely bioresorbable implant material [15-17]. In addition to its space-filling and barrier functions, CS has been investigated as a local delivery vehicle for therapeutic agents, such as antibiotics or growth factors.

The aim of this work was to develop a new triphasic bone cement based on hydroxyapatite (HA), βTCP and calcium sulfate hemihydrate (CSH) and to determine the influence of a liquid phase, used for cement pastes preparation, on physical properties of the final implant material. The cement composition seems to be preferable for required rate and kinetics of the bioresorption process.

2. Materials and methods

A mixture of 60 wt.% of calcium sulfate hemihydrate (CSH) supplied by Acros Organics and 40 wt.% of HT powder constituted the solid phase in the studied bone cement. Biphasic HT powder consisting of 60 wt.% of HA powder and 40 wt.% of βTCP was prepared by the wet method using the following reagents: H_3PO_4 p.a. (POCH), CaO p.a. (POCH) and $Ca(OH)_2$ p.a. (POCH). Powder components were mixed in a Retsch MM400 mill for 5 min. at a frequency of 5Hz. 0.25 wt.%, 0.50 wt.%, 1.00 wt.%, 1.25 wt.%, 1.50 wt.%, 2.00 wt.%. chitosan solutions in 0.3 wt.%, 0.5wt.%, 1.0wt.% acetic acid solutions (Aldrich) and 0.25 wt.%, 0.50 wt.%, 0.75 wt.% methylcellulose aqueous solutions (FLUKA CHEMIKA) as well as distilled water were used as liquids in the preparation of the developed bone cement. Liquid to solid phase ratio (L/P) was constant in all formulations and equal to 0.5 g/g.

In Table 1 starting compositions in studied cement preparations are collected.

Table 1.
Starting compositions in the studied cement preparations

Symbol	Solid phase (P)	Liquid phase (L)	L/P ratio [g/g]
	CSH - 60 wt.% HT - 40 wt.%	Chitosan concentration in 0.5wt.% acetic acid solution [wt.%]	0.50
CHT - 1		0.25	
CHT - 2		0.50	
CHT - 3		1.00	
CHT - 4		1.25	
CHT - 5		1.50	
CHT - 6		2.00	
		Methylcellulose concentration in aqueous solution [wt.%]	
CHT - 7		0.25	
CHT - 8		0.50	
CHT - 9		0.75	
CHT - 10		Distilled water	

Rheological properties of the liquids used in the preparation of bone cements were tested using Brookfield rotary viscometer type DV - II+ at rotary speed values of 100 and 200 [rpm] and shear stresses corresponding to them equal to Dr 22 and 44 [s^{-1}]. Setting times of the obtained cement pastes were determined with the aid of Gillmore apparatus according ASTM C266-04 standard [18]. Porosimetric studies were carried out using mercury Auto Pore IV 9500 Porosimeter (Micromeritics). Open porosity as well as pore size distributions in the hardened bodies were established by mercury porosimetry (MIP). Microstructure of the samples was investigated using scanning electron microscopy (SEM, JEOL 5400) equipped with the EDS attachment for elemental analyses in microareas. Compressive strength was determined using universal testing machine (INSTRON 3345) at a crosshead displacement rate of 1.0 mm/min on cylindrical samples (height: 12 mm, diameter: 6mm). Before the tests, the shaped samples were placed in an incubator (ST1 Pol-Eko) for 7 days in humid atmosphere at the temperature of 37°C.

3. Results of the studies and discussion

Results of rheological tests conducted on the solutions used in the preparation of the developed triphasic bone cement are presented in Tables 2 and 3. Viscosity (η) of the studied chitosan solutions changed from 24.00 mPa·s (for 0.25 wt.% solution) to 2634.50 mPa·s (for 2.00 wt.% solution). Analysis of the obtained results allows concluding that chitosan concentration is the main parameter influencing viscosity of the investigated solutions, whereas change in the acetic acid concentration does not have a significant effect on this parameter. Small differences in viscosity values observed for various concentrations of acetic acid solutions can be caused by changes in the environment temperature during the measurements. Viscosity (η) of 0.75 wt.%, 0.50 wt.% and 0.25 wt.% aqueous methylcellulose solutions changed from 7.20 mPa·s (for 0.25 wt.% solution) to 62.40 mPa·s (for 0.75 wt.% solution). Investigations have shown that viscosity of methylcellulose solution increases as its concentration grows. It should be stressed that viscosity of methylcellulose solutions has been several times lower than that corresponding to the chitosan solutions of the same concentrations (Tables 2 and 3).

Measurements have shown that the shortest setting time (TF=4 min) corresponds to the cement paste prepared using distilled water as the liquid phase - CHT-10 (Table 4). Samples belonging to CHT 1-6 series, obtained with chitosan solutions, exhibit longer setting times than those prepared using methylcellulose solutions (CTH 7-9).

Within a series of specimens obtained with chitosan solutions of various concentrations, the recorded setting time values differ to a small extent. The samples of the lowest chitosan solution concentration (CHT-1, 0.25 wt.%) and those of the highest chitosan solution concentration (CHT-6, 2.0 wt.%) are the exceptions since they show the shortest (TF=7 min) and the longest (F=12 min) final setting times, respectively. In the remaining cases, the initial setting times of cement pastes are in the range of 5-6 min, whereas the final ones - within 10-11 min. For cement formulations prepared using methylcellulose solutions the initial setting time is equal to 4 min, the final - 6-7 min. Concentration of methylcellulose solutions does not influence setting times of cement pastes. Analysis of the obtained results leads to the conclusion that too short final setting time (TF = 4 min) and a narrow setting time interval (ΔT = 1 min) of the cement prepared using distilled water makes this material unsuitable for use as a bone defect filler. Setting time values of the other cement pastes are acceptable from applicational point of view.

Table 2.
Viscosity values η of chitosan in acetic acid solutions determined at various rotary speeds and shear rates (Dr) corresponding to them

Chitosan solution concentration [wt.%]		Viscosity η [mPa·s]			
		Rotary speed 100 rpm	Dr 22 s^{-1}	Rotary speed 200 rpm	Dr 44 s^{-1}
in 0.3 wt.% acetic acid	0.25	24.00		21.60	
	0.50	57.60		52.80	
	1.00	263.90		239.90	
	1.25	496.70		441.50	
	1.50	825.40		722.20	
in 0.5 wt.% acetic acid	0.25	26.40		19.20	
	0.50	67.20		55.20	
	1.00	268.70		244.70	
	1.25	561.50		496.70	
	1.50	556.70		722.20	
	2.00	2634.50		2083.00	
in 1.0 wt.% acetic acid	0.25	24.00		19.20	
	0.50	69.60		62.40	
	1.00	333.50		295.10	
	1.25	566.30		506.30	
	1.50	566.30		513.50	
	2.00	2358.50		1915.00	

Table 3.
Viscosity values η of aqueous methylcellulose solutions determined at various rotary speeds and shear rates (Dr) corresponding to them

Methylcellulose solution concentration [wt. %]	Viscosity η [mPa·s]			
	Rotary speed 100 rpm	Dr 22 s^{-1}	Rotary speed 200 rpm	Dr 44 s^{-1}
0.25	7.20		2.40	
0.50	19.20		16.80	
0.75	62.40		50.40	

Table 4.
Initial and final setting time values of the studied cement pastes

Paste symbol	Initial setting time T_I [min]	Final setting time T_F [min]
CHT - 1	5	7
CHT - 2	5	10
CHT - 3	5	10
CHT - 4	5	10
CHT - 5	6	11
CHT - 6	4	12
CHT - 7	4	6
CHT - 8	4	7
CHT - 9	4	7
CHT - 10	3	4

Based on viscosity of the solutions used for cement preparation as well as on the setting time values of triphasic bone cement, the following liquids have been selected for further studies:

- 0.25 wt.% chitosan solution in 0.5 wt.% acetic acid,
- 0.50 wt.% chitosan solution in 0.5 wt.% acetic acid,
- 1.25 wt.% chitosan solution in 0.5 wt.% acetic acid,
- 0.50 wt.% methylcellulose solution in distilled water (viscosity comparable to that of 0.25 wt.% chitosan solution).

Phase composition analysis has shown that the hardened bone cements contain three phases: calcium sulfate dihydrate (CSD), hydroxyapatite - HAp and whitlockite - βTCP in the amounts of: 63.7 wt.%, 25.8 wt.%, 10.5 wt.%, respectively (independently of the type of the liquid phase applied in the cement preparation) (Fig. 1).

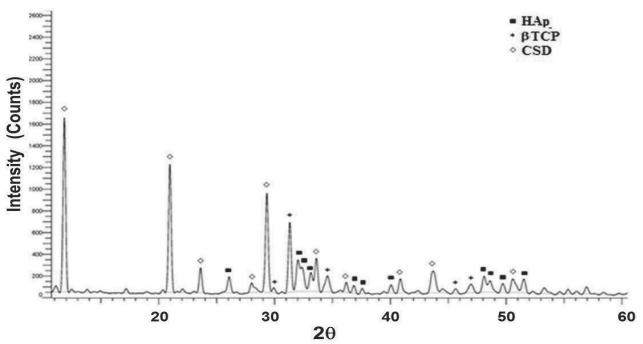

Fig. 1. X-ray diffraction pattern of hardened CHT-2 cement

Open porosity of the hardened bodies has been between ~42% - ~45%, and it has depended mainly on the type of liquid phase used in the cement fabrication. The highest porosity has been observed for the cement obtained using 0.25 wt.% chitosan in 0.5 wt.% acetic acid solution (CHT-1), the lowest for CHT-4 cement, in which 1.25 wt.% chitosan solution has been used as the liquid phase. In Fig. 2 pore diameter distributions in CHT-1 and CHT-4 cements are presented.

Results of mercury porosimetry have shown bimodal pore size distribution in CHT-1 cement. The first maximum, located at 0.45 μm, corresponds to the pores occurring between the needle-like crystals of calcium sulfate dihydrate (CSD). The second maximum at ca. 0.053 μm is characteristic for pores present in agglomerates of calcium phosphate grains. In the case of cement prepared using 1.25 wt.% chitosan in 0.5 wt.% acetic acid solution multimodal (trimodal) pore size distribution has been obtained. The maximum at 0.71 μm indicates the appearance of a new group of pores. They can be connected with the formation of other cement microstructure resulting from another way of CSD grains crystallization caused by the change in viscosity of the liquid phase used in the cement preparation as well as with the space between grain agglomerates connected to each by the polymer layer. The maxima at 0.40 μm and at 0.053 μm are analogous to those appearing in CHT-1 cement. Open porosity of CHT-4 cement is equal to 42%.

Micrographs of the set and hardened CHT-1, CHT-2, CHT-4 cement samples cross-sections have revealed high homogeneity of their microstructures (Fig. 3 a,b,c) in contrast to the cross-section of CHT-8 cement (Fig. 3 d). In the cement obtained using methylcellulose solution non-uniform distribution of the polymer in the material can be observed. Inhomogeneous microstructure may be due to relatively short setting time, which has made homogenization of the paste during its stirring difficult. In the case of materials obtained using both, chitosan and methylcellulose solutions the polymer covered calcium sulfate crystals and filled the space between the grains. Increase in viscosity of the liquid phase inhibited the growth of CSD crystals which resulted in the formation of smaller gypsum needle crystals.

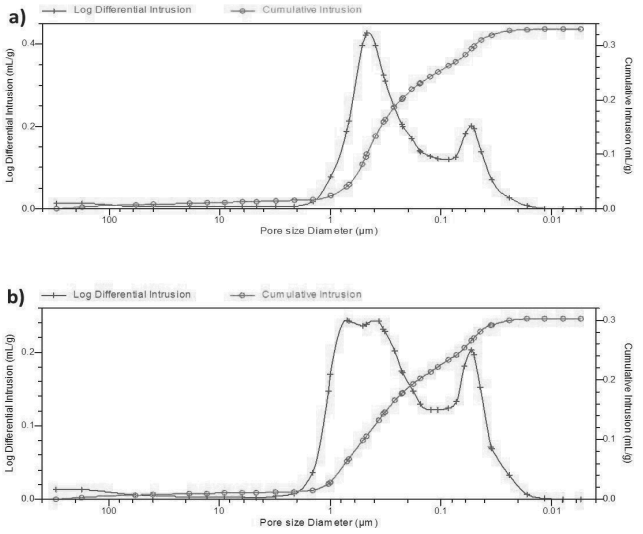

Fig. 2. Dependence of logarithm of partial intrusion on pore diameter for CHT-1 (a) and CHT4 (b) cements

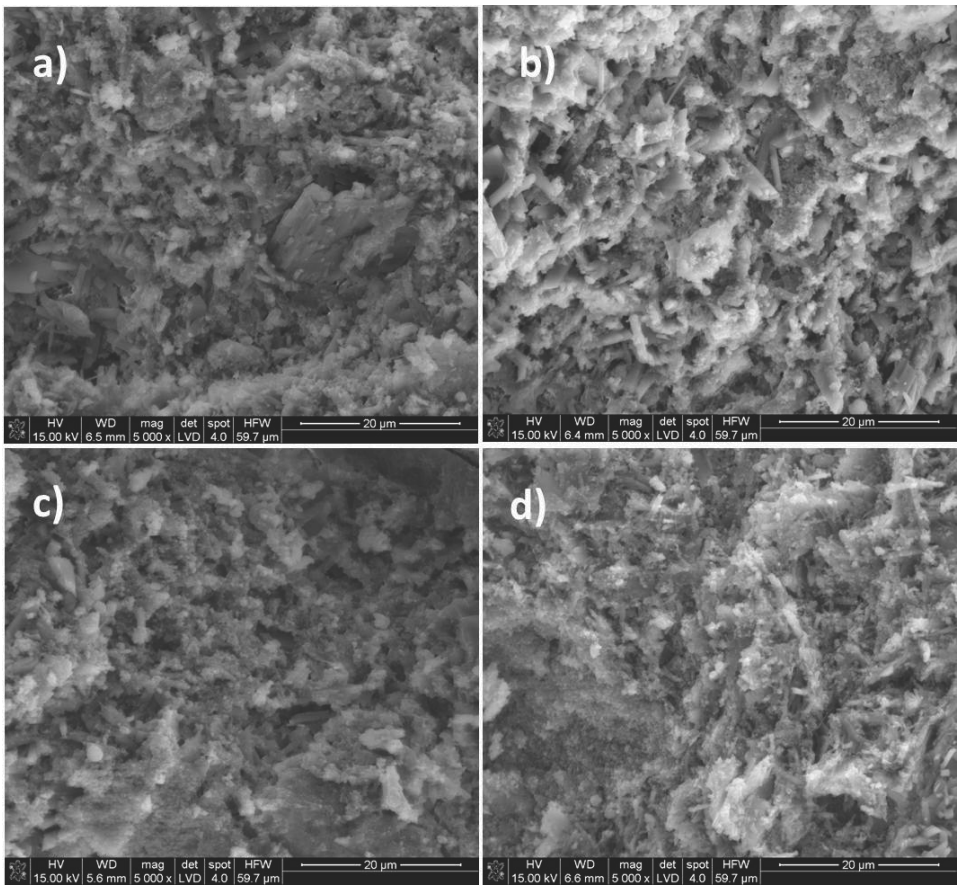

Fig. 3. SEM image of hardened cement pastes a) CHT-1 b) CHT-2 c) CHT-4 (prepared using chitosan solutions) d) CHT-8 (prepared using methylocellulose solution) (magnification: 5000x)

X-ray analysis in microareas (EDS) confirms the presence of calcium sulfate (Fig. 4-1), calcium phosphate (Fig. 4-2) as well as the polymer in the samples (Fig. 4 -1,2).

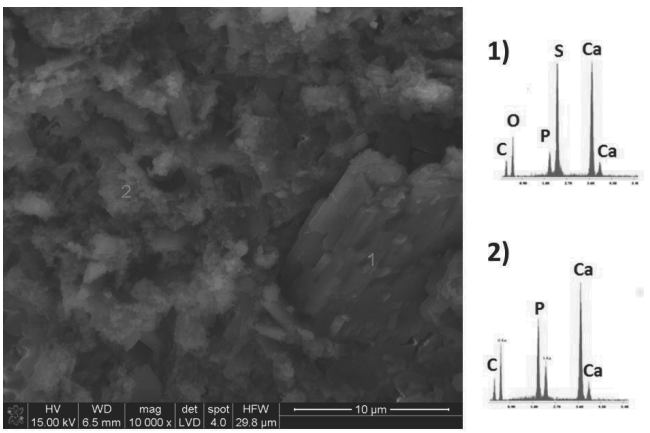

Fig. 4. SEM image of hardened CHT-1 cement paste with marked areas of EDS analysis (magnification: 10000x)

Results of compressive strength studies are presented in Fig. 5.

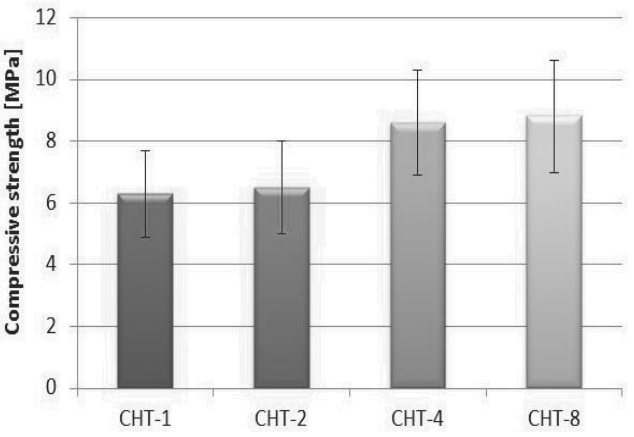

Fig. 5. Compressive strength of phosphate-calcium cements depending on the type and concentration of the liquid phase used in their preparation

The highest compressive strength (8.8±1.8 MPa) exhibit the cement samples prepared using 0.5 wt.% methylcellulose solution in distilled water of viscosity equal to 19.2 mPa·s. Similar compressive strength (8,6±1,7 MPa) has been observed for the cement prepared with 1.25 wt.% chitosan in 0.5 wt.% acetic acid solution of viscosity equal to 561.5 mPa·s. The lowest strength (6.3±1.4 MPa) has been recorded for the hardened cement paste obtained using 0.25 wt.% chitosan in 0.5 wt.% acetic acid solution of viscosity equal to 26.4 mPa·s. Mechanical strength of the studied samples has depended to some extent on the type (higher compressive strength is ensured by application of methylcellulose aqueous solution) and the concentration (the higher chitosan concentration the higher mechanical strength of the sample) of the liquid used in the preparation of the cement paste since these factors influence porosity of the hardened bodies.

4. Conclusions

Triphasic implant cement-type material based on HAp, βTCP and calcium sulfate hemihydrate (CSH) showing high surgical handiness has been obtained. Physico-chemical properties of the studied composite material have been influenced by the type and the concentration of the liquid used in the cement preparation.

Rheological studies have shown that viscosity of the solutions used in the preparation of cement pastes depended mainly on the concentration of chitosan and methylcellulose applied as modifiers. Viscosity increased as the chitosan or methylcellulose concentration grew reaching the maximum value of ca. 2600 mPa·s. In the case of chitosan solution, concentration of acetic acid applied had a small effect.

Cement setting time was influenced by the type of the liquid applied and its viscosity. Cement prepared with distilled water has been characterized by - according to the adopted standards -too short initial setting time which made it unsuitable for use as bone implant material. Materials prepared using methylcellulose solutions exhibited slightly shorter setting time values with respect to those prepared with chitosan solutions.

SEM observations of cement cross-sections have shown that there exists the relationship between the type and the concentration of the solution used in cement preparation and microstructure of the set implant materials investigated. It has been found that the samples prepared using aqueous methylcellulose solution exhibit more inhomogeneous microstructure than the cements prepared using chitosan solutions. Changes in viscosity of chitosan and methylcellulose solutions in comparison to water influenced also gypsum crystallization process. Porosimetric studies have shown that the increase in polymer concentration results in the decrease in material porosity.

Compressive strength was in the range of 6.3±1.4 MPa 8.8±1.8 MPa and was higher for the samples prepared using aqueous methylcellulose solutions.

1.25 wt.% chitosan solution in 0.5wt.% acetic acid and 0.50 wt.% methylcellulose solution in distilled water can be considered as the optimum liquids for the preparation of the investigated HAp, βTCP and CSH - based cements.

Further evaluation of a biological response to the developed materials, including in vitro and in vivo experiments, need to be done.

Acknowledgements

This work has been supported by the project No UDA-POIG.01.03.01-00-005/09-04.

References

[1] W.E. Brown, L.C. Chow, Dental restorative cement pastes, US Patent no. 4518 430, 1985.
[2] T. Kokubo, Bioceramics and their clinical applications, Woodhead Publishing Limited, Cambridge, 2008.
[3] S.V. Dorozhkin, Calcium orthophosphate cements for biomedical application, Journal of Materials Science 43 (2008) 3028-3057.
[4] M. Bohner, Physical and chemical aspect of calcium phosphates used in spinal surgery, European Spine Journal, 10/2 (2001) 114-121.
[5] M. Bohner, Technological issues for development of more efficient calcium phosphate bone cements, A critical assessments, Biomaterials 26/33 (2005) 6423-6429.
[6] F. Bioemers, J. Stahl, M. Sarkar, W. Linhart, U. Rueckert, B. Wippermann, Bone substitution and augumentation in trauma surgery with a resorbable calcium phosphate bone cement, European Journal of Trauma and Emergency Surgery 30/1 (2004) 17-22.
[7] M. Bohner, Reactivity of calcium phosphate cements, Journal of Materials Chemistry 38 (2007) 3980-3986.
[8] M.P. Ginebra, T. Traykova, J.A. Planell, Calcium phosphate cements, Competitive drug carriers for the musculoskeletal system?, Biomaterials 27 (2006) 2171-2177.
[9] G. Daculsi, Biphasic calcium phosphate concept applied to artificial bone, implant coating and injectable bone substitute, Biomaterials 19 (1998) 1473-1478.
[10] R.Z. LeGeros, S. Lin, R. Rohanizadeh, D. Mijares, J.P. LeGeros, Biphasic calcium phosphate bioceramics, Preparation, properties and applications, Journal of Materials Science: Materials in Medicine 14 (2003) 201-209.
[11] S.D. Langstaff, M. Sayer, T.J.N. Smith, S.M. Pugh, Resorbable bioceramics based on stabilized calcium phosphates, Part II, Evaluation of biological response, Biomaterials 22 (2001) 135-150.
[12] G. Daculsi, P. Weiss, J.M. Bouler, O. Gauthier, F. Millot, E. Aguado, Biphasic calcium phosphate/hydrosoluble polymer composites, A new concept for bone and dental substitution biomaterials, Bone 25/2 (1999) 59S-61S.
[13] I. Alam, I. Asahina, K. Ohmamiuda, S. Enomoto, Comparative study of biphasic calcium phosphate ceramics impregnated with rhBMP-2 as bone substitutes, Journal of Biomedical Materials Research 54 (2001) 129-138.
[14] X.D. Zhu, H.J. Zhang, H.S. Fan, W. Li, X.D. Zhang, Effect of phase composition and microstructure of calcium phosphate ceramic particles on protein adsorption, Acta Biomaterialia 6 (2010) 1536-1541.
[15] S.F. Rosenblum, S. Frenkel, J.R. Ricci, H. Alexander, Diffusion of fibroblast growth factor from a plaster of Paris carrier, Journal of Applied Biomaterials 4 (1993) 67-72.

[16] G. Gomez d'Ayala, A. De Rosa, P. Laurienzo, M. Malinconico, Development of a new calcium sulfate-based composite using alginate and chemically modified chitosan for bone regeneration, Journal of Biomedical Materials Research A 81/4 (2007) 811-820.
[17] S.T. Maeda, C.M. Bramante, R. Taga, R.B. Garcia, I.G. De Moraes, N. Bernadineli, Evaluation of surgical cavities filled with three types of calcium sulfate, Journal of Applied Oral Science 15/5 (2007) 416-419.
[18] Standard Test Method for Time Setting of Hydraulic-Cement paste by Gillmore Needles, ASTM C266-04, ASTM Annual Book of standards, West Conshohocken, PA 19428-2959, USA.