

CHARACTERIZATION OF THE NOVEL CALCIUM PHOSPHATE/SULFATE BASED BONE CEMENTS

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Abstract

Calcium phosphate cements (CPCs) are a family of self-setting, bone repair materials. CPCs possess excellent biocompatibility, surgical handiness and adequate mechanical properties but reveal slow resorption *in vivo*. Currently, very interesting group of CPCs are biomaterials composed of α -tricalcium phosphate (α -TCP, α -Ca₃(PO₄)₂) and calcium sulfate. Calcium sulfate hemihydrate (CSH, CaSO₄·0.5 H₂O) possesses a long clinical history in different fields of medicine and is widely recognized as a safe, fast setting and resorbable implant material. The main goal of this study was to investigate how different factors influence the phase composition and physico-chemical properties of the new, cement-type material on the basis of α -TCP, CSH and anhydrous dicalcium phosphate (DCP, CaHPO₄). In presented work two different powder phase compositions and three liquid phases were used to produce new bone substitutes. XRD results showed that obtained materials, after setting and hardening, consisted of α -TCP, DCP, DCPD (CaHPO₄·2H₂O), HA and bassanite phases. Initial (I) and final (F) setting times of the cement pastes were determined with Gillmore needles and differed in the range of 4-14 min (I) and 10-30 min (F). Increase in the amount of CSH in the powder component resulted in shortening of setting time. Microstructure of cements was evaluated on the fractured samples by scanning electron microscopy (SEM) and the porosity via mercury porosimetry. Open porosity of the final materials was similar for all investigated compositions. Compressive strength depended on the composition and increased steadily over a period of maturation. The results obtained suggest that calcium phosphate/sulfate bone cement has the potential to be applied for bone augmentation.

Keywords: biomaterial, calcium phosphate, calcium sulfate, dicalcium phosphate anhydrous

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Introduction

Calcium phosphate-based bioceramics (CaPs), including hydroxyapatite (HA), are an outstanding class of bone grafting materials [1,2]. Calcium phosphate cements (CPCs) constitute a family of promising, self-setting biomaterials, evaluated nearly 30 years ago by LeGeross [3] as well as Brown and Chow [4]. CPCs are considered to be excellent bone void fillers because of the unique combination of osteoconductivity, biocompatibility and mouldability.

Calcium phosphate bone cements are obtained by mixing one or several calcium phosphate powders with an adequate amount of the aqueous solution to produce a shapeable paste, which hardens within a restricted period of time. This kind of materials can perfectly fill a bone cavity even in the most complex shape. Properties of the final material depend on the characteristics of solid and liquid phases, liquid to powder ratio and reaction conditions [1,2,5,6]. A great number of self-setting cement formulations have been developed during the last three decades [1,2,7-12]. Despite of numerous, mentioned above, advantages of calcium phosphate cements, they possess also some drawbacks and for this reason extensive researches are still conducted to develop an ideal, chemically bonded implant material. Currently the main concern of engineers and surgeons is to reach higher resorption rate and improved mechanical properties of CPCs, leading to more efficient bone reconstruction [1,2].

The resorption properties of calcium phosphate based biomaterials are generally believed to be related to the solubility of their constitutive phases. A number of CaPs ceramic and cement compositions have been studied to improve the bioactivity and biodegradation properties [11,12]. The main concept of biphasic calcium phosphate ceramics is aimed to strike the right balance between the high stability of hydroxyapatite and solubility of tricalcium phosphate (TCP, Ca₃(PO₄)₂) phases to control material resorption process [13].

Nowadays, very interesting group of CPCs are biomaterials based on α -tricalcium phosphate (α -TCP) and calcium sulfate. Calcium sulfate hemihydrate (CSH - CaSO₄·0.5 H₂O) is also known as a safe, biocompatible implant material which possesses a long clinical history [1,7,8,14-16]. Despite the numerous advantages, calcium sulfate possesses also some drawbacks. Its main disadvantage is too quick resorption rate which is considered to impede a gradual replacement of biomaterial by the new bone tissue. However, for the same reason calcium sulfate has found a new application and is used as a fast resorbable component of biocomposites, which produce the porosity *in vivo* [8,16,17].

Another interesting approach is combining α -tricalcium phosphate with dicalcium phosphate which during the chemical reaction can create octacalcium phosphate [9,10,12]. Octacalcium phosphate (Ca₈H₂(PO₄)₆·5H₂O; OCP) is more soluble and less stable at physiological conditions than calcium hydroxyapatite [18]. The hydrolysis of OCP to HA is thermodynamically favored and irreversible [19]. Monma et al. [9] obtained OCP via reaction of α -tricalcium phosphate and dicalcium phosphate dihydrate (CaHPO₄·2H₂O; DCPD) in water. The same product was formed in the cement composed of α -TCP and dicalcium phosphate anhydrous (CaHPO₄; DCPA). The hardening time of this CPCs was relatively slow, up to 30 minutes [10]. Synthetic OCP stimulates osteoblastic cell differentiation *in vitro* [20,21].

The aim of our study was to investigate how the initial powder and liquid phase compositions influence, after setting and hardening, the phase compositions, microstructure and compressive strength of the new cement type bone substitutes on the basis of α -TCP, CSH and DCP.

Materials and Methods

The investigated, self-setting composite cements were produced using dicalcium phosphate anhydrous (DCP, POCH, Poland), α -tricalcium phosphate and calcium sulfate hemihydrate (CSH, Acros Organics, USA).

Alpha-TCP powder was synthesized by a wet chemical method using chemical grade $\text{Ca}(\text{OH})_2$ (POCH, Poland) and 85 wt% solution of H_3PO_4 (POCH, Poland). Obtained powder was sintered, ground in an attritor mill to the grain size below 0.06 mm and sieved. Two different powder batches: M-1 and M-2 were produced by mixing α -TCP and DCP (CaHPO_4) with 20 wt% and 30 wt% calcium sulfate hemihydrate ($\text{CaSO}_4 \cdot 0.5 \text{H}_2\text{O}$), respectively (TABLE 1). The weight ratio of α -tricalcium phosphate and dicalcium phosphate in the powder batches was fixed and equal 7:3. Mixing process was performed using the Retsch MM400 mixer mill (5 Hz/5 min). Distilled water and Na_2HPO_4 solutions (2.0 wt% and 2.5 wt%) were applied as liquid phases in pastes preparation.

TABLE 1. Initial powder composition of the studied cements.

Cement	α -TCP [wt%]	DCP [wt%]	CSH [wt%]
M-1	56	24	20
M-2	49	21	30

Setting times

Initial (I) and final (F) setting times of obtained cement pastes were determined using the Gillmore needles according to ASTM C266-04 specification (3 samples per each measurement) [22]. The appropriate amounts of powder (P) and liquid (L) phase were mixed in a mortar for 1 min to produce mouldable, self-setting materials. Liquid to powder ratio (L/P) was the same for both cements, namely 0.44 ml/g. All experiments were performed at $23 \pm 2^\circ\text{C}$.

Phase composition

Phase composition of the hardened cement bodies was characterized by X-ray diffraction method using $\text{CuK}_{\alpha 1}$ radiation, Ni-filter and the standard Bragg-Brentano geometry. Data were collected in the 2θ angle range of 10 – 90° with a step size of 0.002° (Philips diffractometer, X'Pert Pro). The Rietveld method was used for the quantitative phase analysis.

Microstructure

Microstructure of the fractured set cements was measured by scanning electron microscopy (SEM, Nova NanoSem 200). Chemical compositions in microareas were determined using Energy Dispersive X-Ray Spectroscopy (EDS, Model 4457F_ISUS-SN). All samples were carbon-sputtered to prevent surface charging effects.

Porosity

Mercury intrusion porosimetry (MIP, Autopore IV Micro-meritics) was applied to determine the open porosity and pore size distribution within the cement samples.

Compressive strength

The compressive strength of set cements was evaluated as the average value of 10 cylindrical specimens (6 mm in diameter and 12 mm in high), tested under compression at a cross-head speed of 2 mm min^{-1} in the Universal Testing Machine (Instron 3345). Mechanical tests were performed: 1, 7, 14 and 28 days after setting. Samples for compression test were stored at 37°C .

Results and Discussion

Setting times

The initial and final setting times of the obtained materials differed in the range from 4 to 7 min (I) and from 10 to 30 min (F) (TABLE 2). The setting times strongly depended on the concentration of Na_2HPO_4 in the liquid phase.

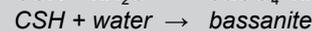
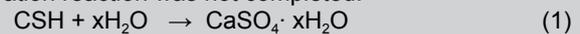
TABLE 2. Setting times of studied cement compositions.

Liquid phase	Material	L/P [ml/g]	Initial setting time I [min]	Final setting time F [min]
Distilled water	M-2	0.44	14	30
2.0 wt% Na_2HPO_4	M-1	0.44	7	22
	M-2		6	18
2.5 wt% Na_2HPO_4	M-1	0.44	4	10
	M-2		4	13

The setting time obtained when distilled water was used as the liquid phase reached 30 minutes and was too long from the clinical point of view. Increase in the concentration of Na_2HPO_4 in the solution resulted in faster setting process of CPC pastes. Values achieved when 2.5 wt% Na_2HPO_4 was used were found to be the most suitable for future applications.

Phase composition

Results of XRD analysis (FIG. 1) revealed presence of bassanite phase in both materials and indicated that the hydration reaction was not completed.



Calcium sulfate hemihydrate reacted with the aqueous solutions (equation 1) and created not fully hydrated calcium sulfate: $\text{CaSO}_4 \cdot x\text{H}_2\text{O}$, where $0.5 \leq x < 2.0$. Presumably presence of α -TCP phase in the initial cement composition influenced hydration process of calcium sulfate by formation of a calcium phosphate layer on the calcium sulfate crystals. This process could lead to the passivation of their surface and impede the reaction with water.

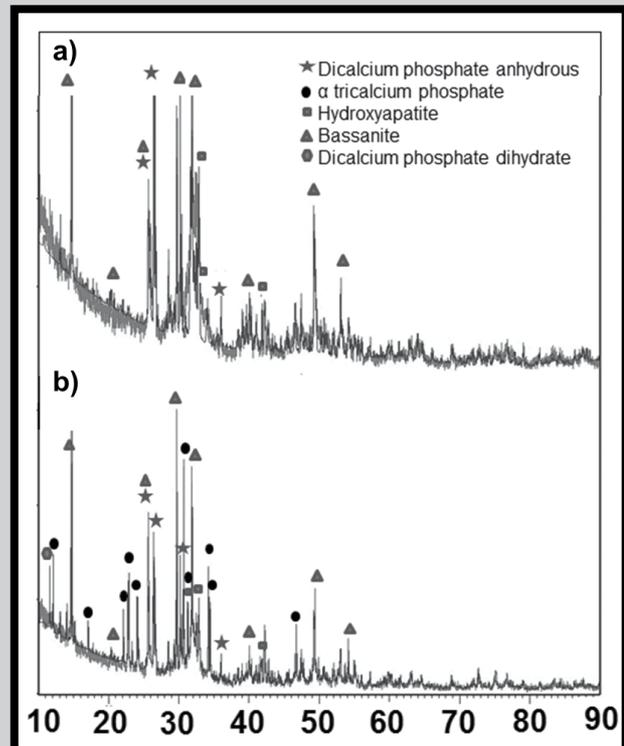


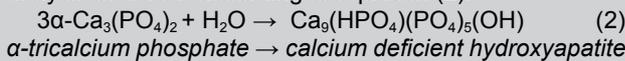
FIG. 1. XRD patterns of the set and hardened cements: a) M-1 and b) M-2.

After setting studied cements composed also with hydroxyapatite and anhydrous dicalcium phosphate (DCP). Alpha-TCP and small amounts of brushite (DCPD, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) phase occurred in the case of cement M-2 (TABLE 3).

TABLE 3. Phase composition of the studied cements 4 weeks after setting.

Cement	HA [wt%]	Bassanite [wt%]	α -TCP [wt%]	DCP [wt%]	DCPD [wt%]
M-1	51	17	0	32	0
M-2	13	33	26	26	2

Presence of DCP in the set and hardened body indicates on the high stability of this phase and very limited reaction with water as well as with α -TCP. Hydroxyapatite phase is created not as expected via conversion from intermediate phase (octacalcium phosphate), but by the process of hydrolysis of α -TCP according to equation (2).



This observation stays in agreement with researches conducted by Fernandez et al. [23]. Results of his studies showed that for the cement composed of α -TCP and DCP mechanism of setting did not involve the reaction between constituents. Probably kinetics of setting process is the reason why DCP remained in the final material.

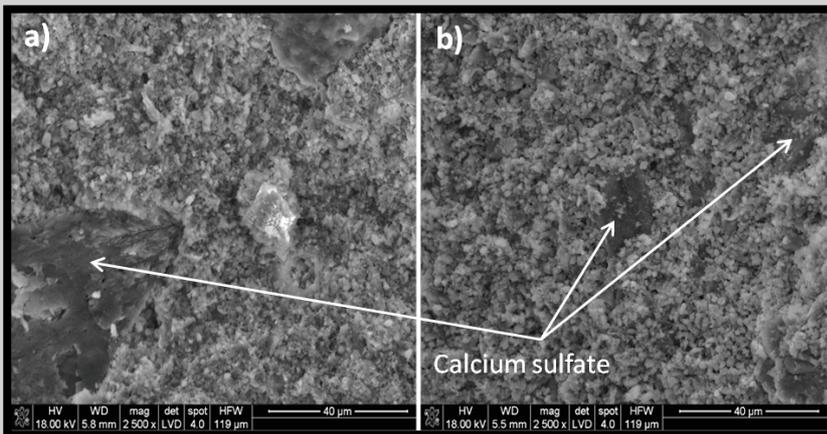


FIG. 2. SEM images of fractured hardened cement bodies: a) M-1, b) M-2.

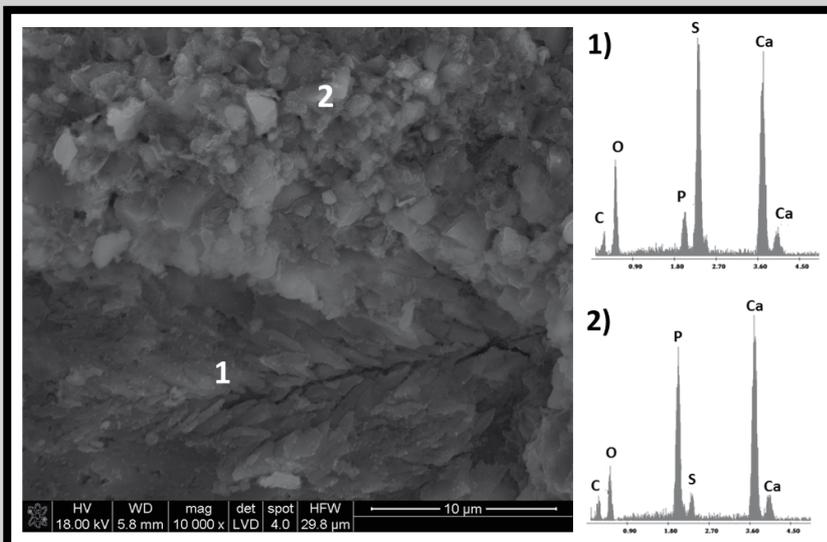


FIG. 3. EDX analysis of the crystals on the fractured surface of the M-1 sample.

Fernandez explained the lack of DCP reactivity by a blocking effect in the dissolution of this compound due to a process of heterogeneous nucleation.

In the case of cement M-1 the whole amount of tricalcium phosphate phase completed hydrolysis process whereas for cement M-2 as much as 26 wt% of α -TCP remained unreacted in the material. Presence of higher amount of calcium sulfate in the cement M-2 seemed to retard hydration process of α -TCP to hydroxyapatite. These results stayed in the agreement with the studies performed by Durucan and Brown [24] as well as Czechowska et al. [25] who confirmed that presence of CSH delayed hydroxyapatite formation.

Microstructure

The scanning electron micrograph of the fractured cement samples are presented in FIG. 2.

SEM measurements showed that the microstructure was composed mainly with the tightly entangled calcium phosphate crystals. The compact matrix, created by calcium phosphate grains provided a mechanical rigidity to the cement. The large calcium sulfate crystals (bassanite) were present in the form of blocks and plates embedded in the calcium phosphate matrix. No interconnected calcium sulfate needle-like crystals, characteristic for calcium sulfate dihydrate could have been noticed on the fractured surfaces. EDS results confirmed presence of calcium sulfate and calcium phosphate phase in the set cement samples (FIG. 3).

Porosity

Mercury porosimetry analysis of the set cement samples revealed a bimodal pore size distribution (FIG. 4).

Open porosity values for the M-1 and M-2 cements were similar and equal 44% and 47%, respectively. Both materials possess pore size diameter ranging from 5 nm to around 1 μm . While interpreting obtained data we should be aware that mercury intrusion porosimetry has limitations when applied to materials with irregular pore geometry. For such materials MIP does not measure the true distribution of sizes for pore geometries found in the samples. If large internal pores are connected by narrow throats the mercury porosimetry will misrepresents their size and show just the diameter of throats [26]. This is known as the 'ink bottle' effect. The larger misfit between intrusion and extrusion curves the stronger influence of ink bottle effect on the results of porosity measurements. In the case of studied cements a small misfit between the curves is observed (FIG. 4 c,d). what indicates that also larger pores can be present in the hardened cements' bodies.

Two different kinds of pores were found in examined materials (FIG. 4). First, lower and wider peak (max. ~ 30 nm), was connected with voids between calcium phosphate grains, whereas the second, more narrow one (max. ~ 0.25 μm) may be assigned to the population of bigger pores present between agglomerates of CaPs grains. The bigger pores can be also connected with the presence of voids between calcium phosphate and sulfate crystals.

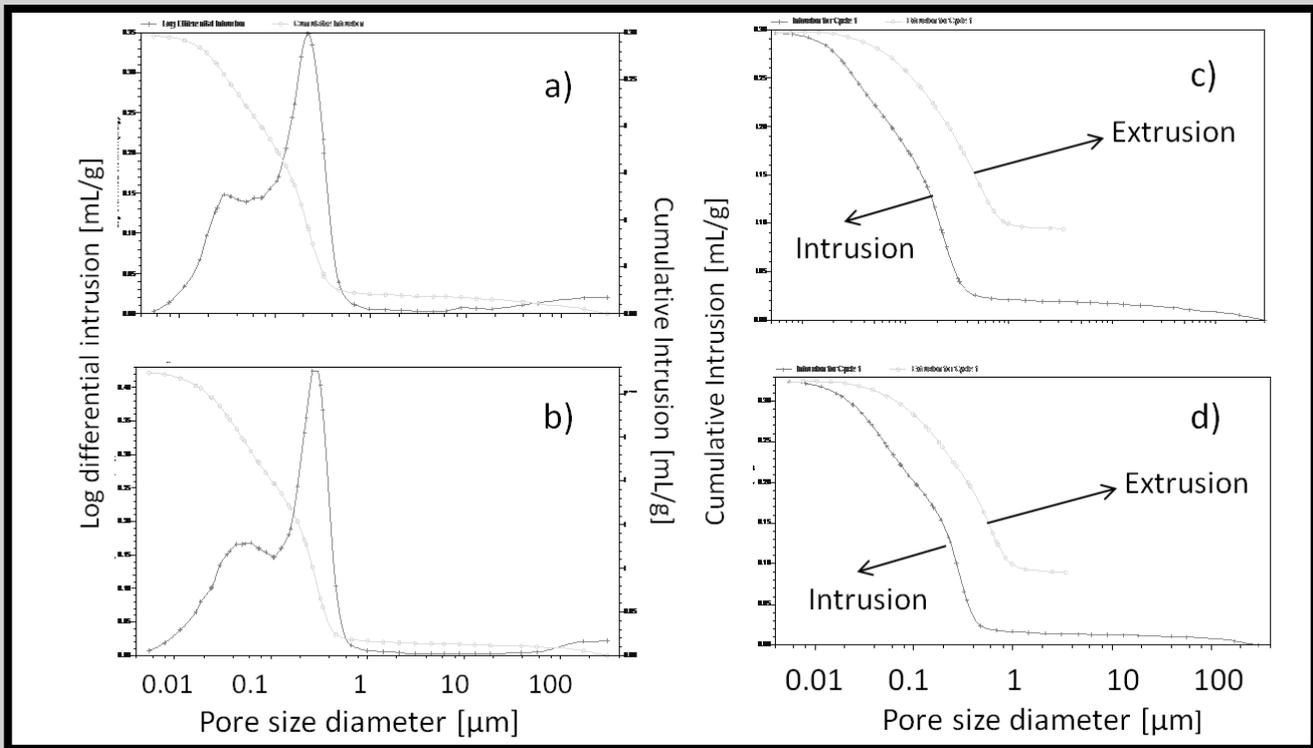


FIG. 4. Pore size distribution curves of examined cements: a) M-1, b) M-2. Curves representing intrusion and extrusion process: c) M-1, d) M-2.

Compressive strength

The results of compressive tests performed on the cement samples are presented in FIG. 5.

As expected the chemical composition of examined cements as well as maturation affect their mechanical properties. The compressive strength of obtained materials increased during the setting process and ranged from 4.1 MPa to 9.4 MPa for cement M-1 and from 6.7 MPa to 8.8 MPa for cement M-2. The amount of calcium sulfate in the initial powder composition of cements slightly influenced mechanical properties of the final samples. Material with 30 wt% of calcium sulfate possessed higher mechanical properties at the beginning of the experiment (after 1 and 7 days), however after 14 and 28 days compressive strength values were comparable for both materials. The setting time of commercially available CPCs is typically close to 10 min, however the setting and hardening reaction within the cement body take much longer - usually a couple of days. The influence of this process is visible in FIG. 4. Furthermore conversion of tricalcium phosphate into HA progresses in time, what simultaneously with process of hardening improved mechanical strength.

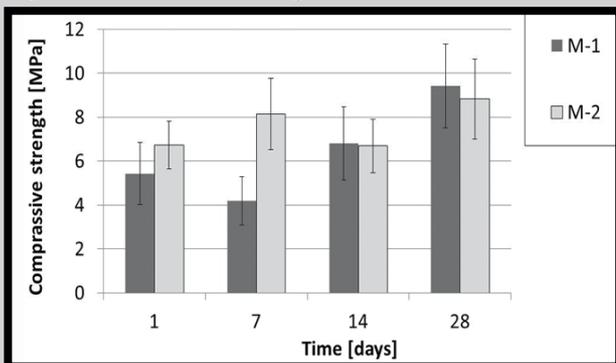


FIG. 5. Compressive strength of examined cement materials as a function of time.

Conclusions

1. The new, gradually resorbable bone cements based on α -tricalcium phosphate (α -TCP), anhydrous dicalcium phosphate (DCP) and calcium sulfate hemihydrate (CSH) were developed.
2. From the point of view of future medical applications the amount of 30 wt.% of CSH in the powder phase was established as the superior one.
3. Disodium hydrogen phosphate (Na_2HPO_4) is an effective accelerator of the α -TCP setting reaction. 2.5 wt% solution of Na_2HPO_4 was found to be a liquid phase of choice in the case of examined materials.
4. The obtained cements possessed after setting and hardening bimodal pore size distribution with the pore diameters ranged from 5 nm to 1 μm .
5. Compressive strength of examined composite materials after 28 days reached up to ~9 MPa.
6. The obtained cement-type bone substitutes revealed good surgical handling and may be applied in filling bone defects.

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