Silver Containing Calcium Phosphate Bone Cement With Ag/Ca Atomic Ratio Equal To 0.05: Synthesis And Characterization

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Abstract

The introduction of silver into calcium phosphate bone cement (CPC) increases the strength and antimicrobial activity of the material. In this study, we synthesized silver-containing CPC by introducing silver phosphate salt Ag_3PO_4 in the cement powder with a Ag/Ca atomic ratio equal to 0.05. α-Tricalcium calcium phosphate (α-TCP, $Ca_3(PO_4)_2$) and Hydroxyapatite (HA) were mixed with dicalcium phosphate dihydrate (DCPD, $CaHPO_4.2H_2O$) to form the cement powder. Deionized water (DI) solution was used as the cement fluid. CPC based on silver containing apatite phase was obtained. The phase composition and setting time of the cement was determined. The formation of apatite was verified by XRD and FTIR analysis, the initial and final setting times of the cement are 11 and 35 min.

Keywords: Calcium Phosphate Cement, Silver, Setting Time.

Introduction

Over the years, reconstructive surgery has increasingly relied on the use of synthetic bioresorbable materials, such as calcium phosphates [1] and biodegradable polymers, to replace autografts or bone material of animal origin [2]. Calcium phosphate cements (CPCs), such as those made from α - and β-tricalcium phosphate, are known for their osteoconductivity and resorbability properties and are commonly used as bone substitute materials. The CPCs can set to form a matrix of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HA) at a pH value of > 4.2 or brushite ($CaHPO_4.2H_2O$) at a pH value of ≤ 4.2 [3]. HA cements have generally attracted more interest as they can exhibit a composition comparable to that of the mineral component of natural bone [4]. Postoperative infections are one of the largest problems in orthopaedic surgery and such infections often lead to severe pain, loss of bone tissue and possibly removal of implants, which consequently requires a second operation [5–7]. These orthopaedic-related infections are largely caused by Gram-positive bacteria such as Staphylococcus aureus (S. aureus) [8]. These bacteria are treated using various regimes of antibiotics and, for this reason, an increasing number of these bacteria have become resistant to antibiotics, which are commonly found in infections caused due to orthopaedic operations [9, 10]. In order to reduce the incidence of implant-associated infections, several biomaterials have been proposed [11, 12]. On the other hand, silver has been widely used in medical devices as a broad-spectrum antimicrobial agent with low toxicity [13]. In particular, an antibacterial effect was observed on S. aureus, which is the most frequent infective agent after prosthesis implantation [14, 15]. Among the developed medical devices, silver was introduced by different methods into apatitic matrices during apatite synthesis, for example by co-precipitation of calcium hydroxide or calcium salt and silver nitrate with a phosphate compound, by direct formation of silver-doped hydroxyapatite (Ag-HA) coatings, or by an additional step of impregnation of the hydroxyapatite (HA) gel or HA coating in a solution of $AgNO_3$ [16–19]. The present study focuses on the association of silver, in the form of Ag_3pO_4, with a cement powder in order to confer the antibacterial activity of this cement. The objective of this study was to evaluate the physical–chemical properties of such silver-loaded cement.

Materials and Methods

Synthesis of α-TCP, HA, DCPD and Ag_3PO_4

The α-tricalcium phosphate, nanocrystalline hydroxyapatite and Ag_3PO_4 were synthesized and characterized as described in our previous publications according to methods described in [20], [21] and [22], respectively. The dicalcium phosphate dihydrate used in this work was prepared at room temperature by the rapid addition of solution A to the stirring solution B with a molar ratio Ca/P = 1.0. Solution A was prepared at room temperature by the rapid dissolution of calcium nitrate tetrahydrate ($Ca(NO_3)_2$, $4H_2O$) (Scharlau, Spain) in distilled water. Solution B was prepared at room temperature by the rapid dissolution of diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (Riedel-de Haën, Germany) in distilled water at a pH of 4.5-5. The Ag_3PO_4 sample prepared by directly mixing $AgNO_3$ aqueous solution with $(NH_4)_2HPO_4$ solution under dropwise addition followed by vigorous stirring. Typically, 0.408 g $AgNO_3$ was first dissolved in 20 mL (0.12 M) (DI) water. Then, a uniform $(NH_4)_2HPO_4$ aqueous solution (0.04 M) was formed by mixing 0.108 g $(NH_4)_2HPO_4$ with 20 mL DI water and was dropwise injected to the above solution under magnetic stirring during 2 h in the dark. The as-synthesized yellow precipitate was separated by filtration, washed several times with DI water. Finally the sample was dried at 80°C for 12 h.

Cement preparation

The CPC investigated in this study are of the calcium-deficient HA, (CDHA) type. Its powder consists of α-tertiary calcium phosphate (70% α -TCP), nanocrystalline HA (5% HA) and dicalcium phosphate dihydrate (25% DCPD) with a molar ratio Ca/P = 1.38. This small amount of HA in the starting powder acts as a seed material and accelerates the reaction with the hardener [23]. Silver was introduced into the cement in the solid phase by means of silver salts (Ag_3PO_4); the silver salt was introduced in the cement formulation in order to reach a Ag/Ca atomic ratio of 0.05. The powder phase was mixed with DI water until a homogeneous paste obtained at room temperature.

Characterization

Setting time

Powder phase components were placed into an agate mortar. A volume (ml) of the DI water is dropped onto the powder and the mixture is kneaded with an agate pestle for 2 min thoroughly to form a homogenous paste. The powder phases were mixed with DI water at a liquid to powder ratio L/P = 0.4 ml/g.

The so-called Gillmore needles are suitable to measure the setting times of CPCs. The light and thick needle are used to measure the initial setting time, t_i the heavy and thin needle for the final setting time, t_f. The clinical meaning of t_i is that it indicates the time from where the paste may not be deformed without damaging the structure of the solidifying cement. That of t_f indicates the time from when the cement can be touched without scratching it. These parameters are important because the cement must be applied before t_i and the wound may be closed after t_f. Further, the setting characteristics and the strength are considerably improved when going from room temperature to body temperature.

The cement was soaked in distilled water at 37°C for 0, 1, 7 and 30 days

- ✓ ***Cement characterization***

The cements were studied by powder X ray diffraction on a Diffractometer system XPERT-3 PW3050/60 in a theta-theta setup with Cu-Kα irradiation, nickel filter. Diffraction patterns were collected between angles (2θ) of 10–60°, in steps of 0.02° with 1 s per step. The microstructure of materials was studied by scanning electronic microscopy on a ESEM, Quanta 200 FEI at operating voltage of 15kV; The Silver containing calcium phosphate bone cement samples were analyzed without deposition of a conductive layer on their surface.. The cements

were diluted in KBr and FTIR analysis was carried out on a VERTEX 70, Genesis Series FT-IR spectrometer with a scanning range from 450 to 4000 cm^{-1} and resolution of 2 cm^{-1}.

Results and Discussion

The XRD patterns of cements before and after soaking in distilled water at 37°C for 1, 7 and 30 days are shown in Figure 1. It appears that a poorly crystallised apatite (CDHA) was formed during the setting reaction by the consumption of brushite and α-TCP. Nevertheless, some Tricalcium phosphate, initially introduced, remained in the final composition. The addition of silver as Ag_3PO_4 into the solid phase did not affect the final composition.

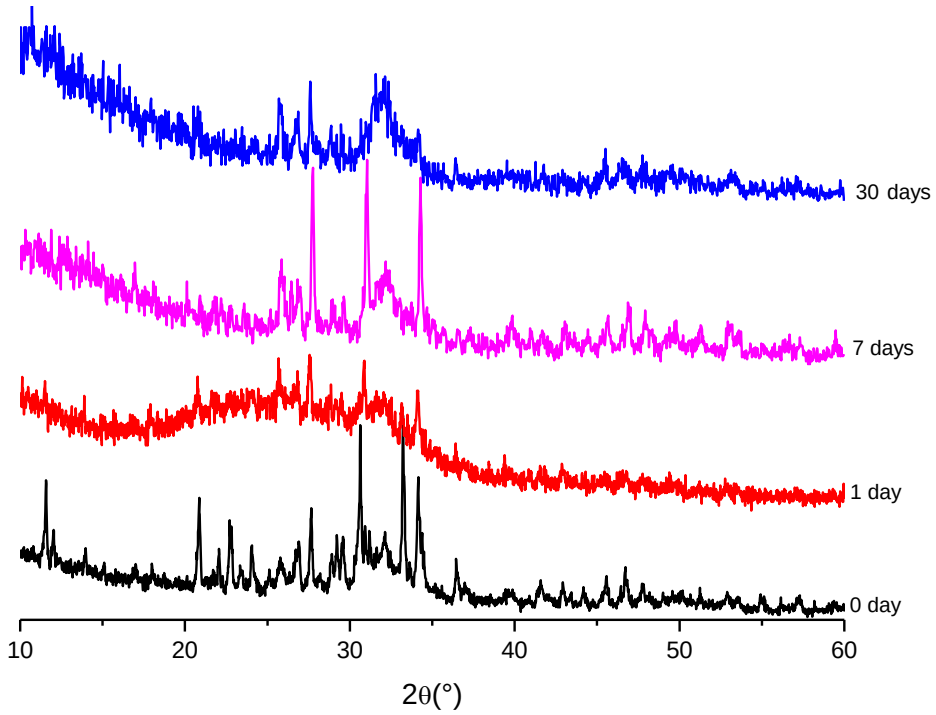


Figure 1. The XRD patterns of cement after soaking in water for: 0, 1, 7 and 30 days

Indeed, the formation of apatite was also verified on FTIR analysis since the absorption bands present in samples spectrum (Figure 2) are characteristic of apatite as displayed on Table 1. We could clearly observe in Figure 2 that at 1 day of soaking in water the decrease in the characteristic band of the brushite phase at 529 cm^{-1} in favour of an increase in apatite characteristic band at 602 cm^{-1}.

Table 1. Wave numbers for the functional groups of TCP

Functional	**ν1**	**ν2**	**ν3**	***ν***		
groups	(PO_4^{3-})	(PO_4^{3-})	(PO_4^{3-})	(HOH)	HPO_4^{2-}	Apatitic PO_4^{3-}
ν(cm-1)	968	559	1043	1633	529	602
			1078	3450		

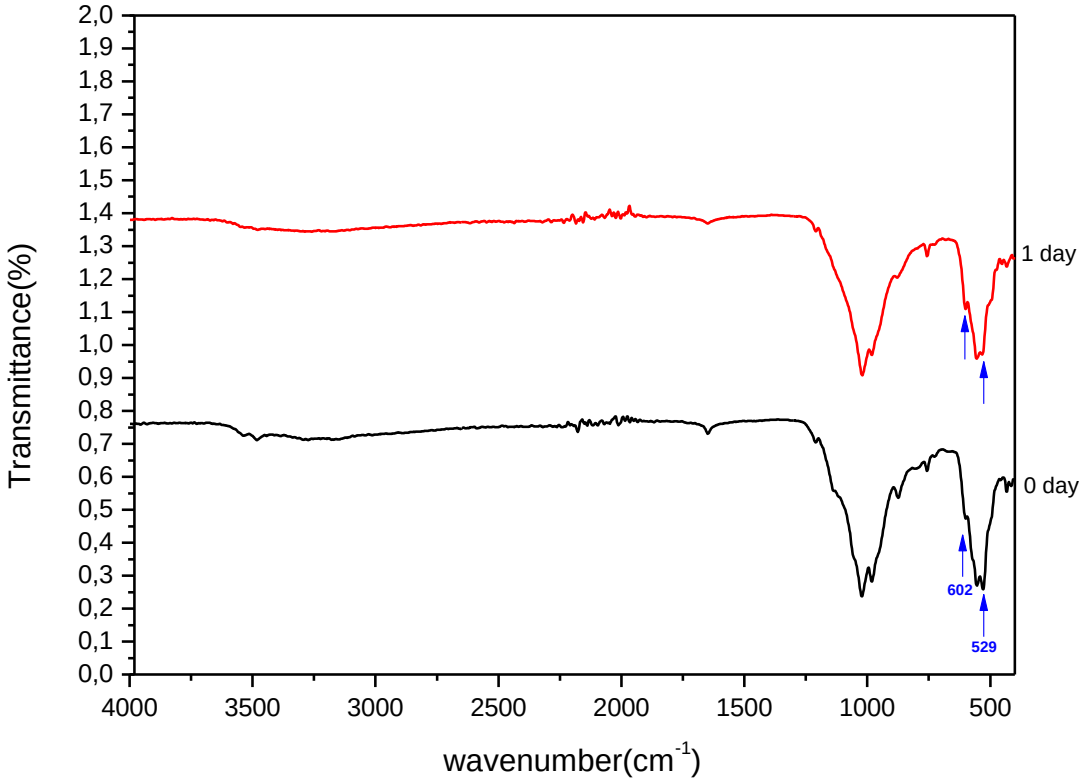
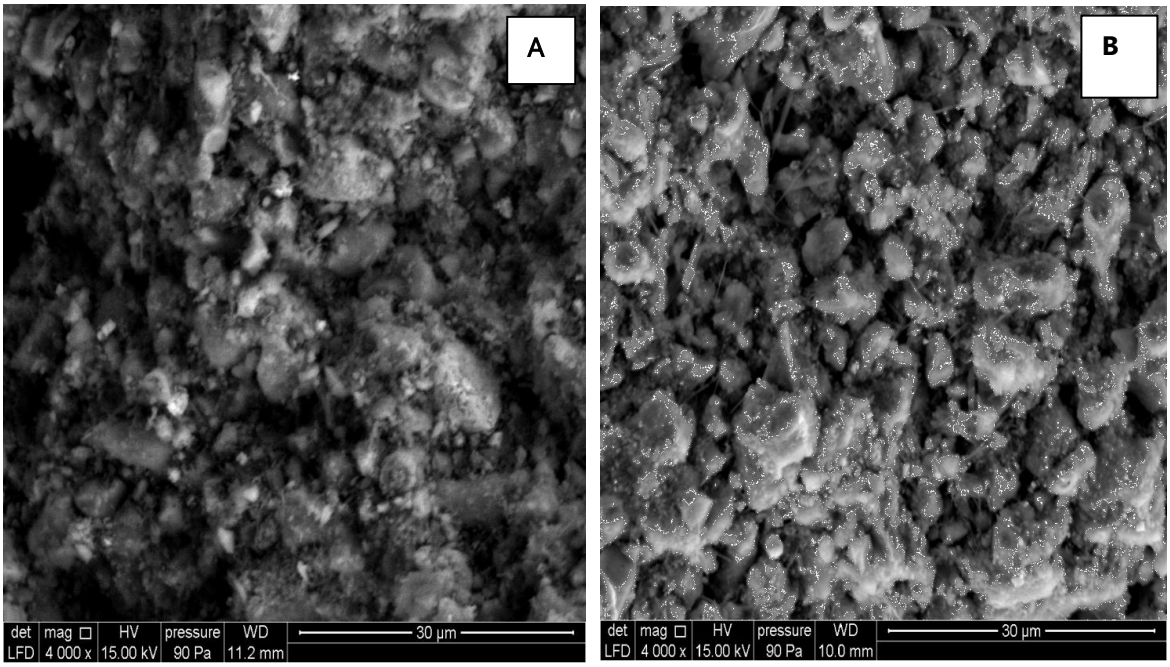


Figure 2. The **FTIR spectrum of cement after soaking in water for: 0 and 1 day**

The SEM images of silver cements are presented in Figure 3. Particles containing silver, which is the heaviest element in the cement compositions tested, appeared much brighter than the calcium phosphate phase. The silver orthophosphate particles were homogeneously distributed throughout the cement matrix.



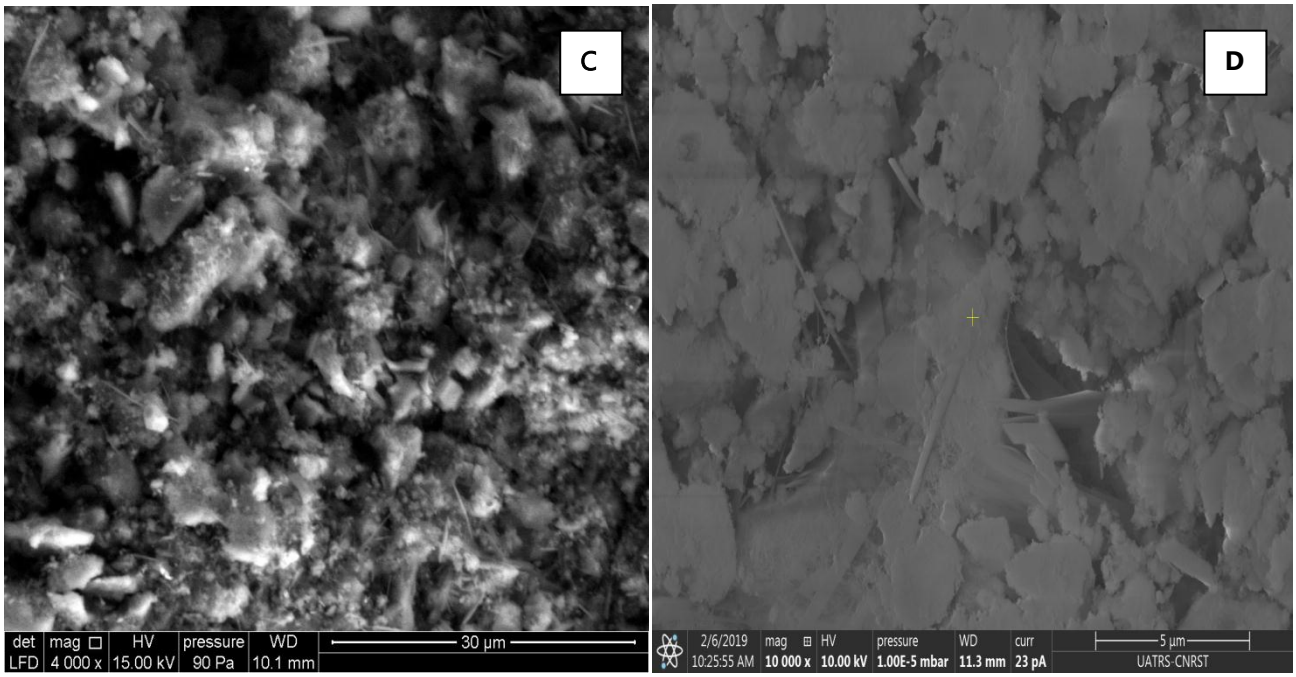


Figure 3. The SEM micrographs of fractured surfaces of cements soaking in water for: (A) 0 day, (B) 1 day, (C) 7 days and (D) 30 days

The values obtained for the initial and final setting times of the cement prepared at liquid-to-powder ratio L/P = 0.4 and Ag/Ca molar ratio equal to 0.05 are 11 and 35 min.

Conclusions

It was possible to synthesize Silver containing calcium phosphate bone cement with Ag/Ca atomic ratio equal to 0.05 by mixing α-$Ca_3(PO_4)_2$, $Ca_{10}(PO4)_6(OH)_2$, $CaHPO_4.2H_2O$ and Ag_3PO_4. Cement setting reaction take place with a relatively fast setting time. Nevertheless, the TCP → apatite conversion was not complete even after 7 days of soaking in distilled water at 37°C, some Tricalcium phosphate, initially introduced, remained in the final composition as shown by the x-ray spectrum.

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