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Modulation, characterization and bioactivity of new biocomposites based on apatite

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Abstract

The present study is focused on preparation of hydroxyapatite (HA)/calcium aluminate (CA) composites and studying the effect of CA content on their mechanical and bioactivity properties. HA/CA composites containing varying CA content (5, 10, 15 and 20 wt.%) were fired at 1250 and 1350 °C to evaluate the extend the stability of HA at high temperatures. The composites were assessed by using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), Vickers micro-indentation (H_v) and cold crushing strength (CCS). Bioactivity testing study was carried out for these composites in simulated body fluid (SBF) for 7 days to confirm the formation of apatite layers onto the surfaces. The results confirmed that the addition of CA to HA improve the thermal stability and the mechanical properties of the composites, especially those composites fired at high temperatures. Also, FT-IRRS and SEM confirmed the formation of bone-like apatite layer on the surface of the composites especially those containing high CA content at both firing temperatures. The stability of HA at high firing temperatures was improved via the addition of CA content. Also, the surface reactions of the composites having high CA content at both firing temperatures were higher than those having low CA content post-immersion for 7 days. Conclusions prove that the HA matrix containing 20 wt.% of CA as in C4 composite could be studied in vivo study in the future for using it as bone substitutes, especially in load bearing sites.

Keywords: Hydroxyapatite; Calcium aluminate; Bioactivity; SBF; FT-IRRS

1. Introduction

An adequate approach in selecting the implant needs a material with similar composition to the mineral component of human bone. There are many bioceramic materials such as hydroxyapatite (HA), tricalcium phosphate (TCP), calcium aluminate (CA) and bioactive glasses (BG) which have been used as fillers to repair bone defects [1]. Calcium phosphate ceramics loaded with bone growth factors are of great value for developing biomaterials [2]. In recent years, considerable attention has been given to the composites which would remain in place after implantation and provide an agent which would encourage osteogensis [3]. The application of calcium phosphate ceramics as materials for bone regeneration has been increasing in different fields such as the bone cement for the self-setting apatite cement which discovered by Brown and Chow [4]. Moreover, they are being used as materials for scaffolds for bone tissue engineering [5,6].

Calcium aluminate was used as dental restorative material [7]. The thermal expansion coefficient of calcium aluminate was determined and was found to be close to tooth substance [8]. The biocompatibility of two variants of accelerated Portland cement investigated in vitro by observing the cytomorphology of osteosarcoma cells in the presence of test materials and the effect of these materials on the expression of markers of bone remolding. The results of this study provide evidence that Portland cement is non-toxic and may have potential to promote bone healing and can produce as viable dental restorative and orthopedic materials [9].

Development of HA based biocomposite materials could be one way to overcome the poor mechanical properties of dense HA. Indeed, the incorporation of a second phase dispersed in a ceramic matrix improves the mechanical reliability [10]. Some attempts to toughen HA by treating with titania or zirconia particles have been studied [11]. AlCaP ceramics were reported to combine all of the best features and are unique in that they

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pride a multipurpose crystallographic system where one phase is resorbed more rapidly than the others. Phases involving calcium and phosphorus are readily resorbed while others contain aluminate are less rapidly resorbed and act as a relatively more stable framework for the development of new bone [12]. Although, HA is chemically similar to the inorganic part of bone, it cannot be used as an implant material for load-bearing application due to its poor mechanical properties. Considerable efforts were made to improve the mechanical properties of HA by the composite formation. The combination of material properties, high strength and toughness of the additives along with the bioactivity of HA, results in a bioactive implant material with improved mechanical properties. Alumina is one of the most widely investigated reinforcement materials for HA bioceramics. The decomposition of hydroxyapatite to tri-calcium phosphate is thought to be an undesirable action, but recently there has been growing interest in developing biphasic calcium phosphate ceramics as they are more effective in bone repair or regeneration [13]. However, the presence of calcium aluminates and its influence on bioactivity and mechanical properties should be investigated.

The present work is concerned with the preparation of hydroxyapatite (HA)/calcium aluminate (CA) biocomposites to be used as dental restorative materials or for orthopedic applications especially in load bearing sites. The composites were fired at 1250 and 1350 °C and characterized by XRD and FT-IR along with the mechanical properties. Also, the bioactivity behavior was conducted for the prepared composites in SBF for 7 days to confirm the formation of carbonated apatite layer onto their surfaces and its enhancement.

2. Experimental

2.1. Preparation of biocomposites

Hydroxyapatite (HA) was prepared according to the specifications of Ergun et al. [14]. The calcium aluminate (CA) was prepared according to Mohamed and Sharp [15]. HA/CA biocomposites (C) were prepared using HA with CA powder in the ratios of 5, 10, 15 and 20 wt.% of CA as demonstrated in Table 1. HA/CA powders having grain size = 80 μm were mixed thoroughly using alcohol, dried, pressed into discs (13 mm diameter) and then fired at 1250 or at 1350 °C.

2.2. Characterization of biocomposites

Phase compositions were assessed using Diano X-ray diffractor USA, Co K_a. $\lambda = 1.79026$ Å, with scanning speed of 2° in 2θ min^{-1} at 36 kV and 16 ma. The infrared spectra of the prepared samples were obtained in the range 4000–400 cm^{-1} using KBr technique by using Fourier transformer infrared spectrophotometer (FT-IR) NEXAS 670, Nicolet, USA. The Vickers micro-hardness (H_v) test was used at a constant load of 500 g for 15 s on polished composites. The measurement of cold crushing strength (CCS) was carried out with cylindrical samples which were subjected to direct compression between two steel plates in a load-controlled testing machine in accordance with PEZ 1979, Machina KN, Germany. The CCS is a complex characteristic reflecting preparation and testing method, specimen geometry, and material properties. Hence, it is influenced by many factors, as indicated by Stroeven [16]. The average value of three samples was taken for the determination of H_v and CCS tests.

Table 1
The chemical composition of the HA/CA biocomposites

	Notation			
	C1	C2	C3	C4
HA (wt.%)	95	90	85	80
CA (wt.%)	5	10	15	20

2.3. Bioactivity behavior

The bioactivity of the composite materials was evaluated by an in vitro study. In vitro bioactivities studies are much cheaper and quicker than in vivo studies. They are also useful in both designing new biomaterials and improving the existing materials for better bioactivity. In order to study the bioactivity, samples were soaked in SBF, proposed by Kokubo et al. [17] at body temperature (37 °C) and pH 7.4 for several periods [18]. The SBF was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$ and Na_2SO_4 in bi-distilled water. The pH of the solution was adjusted to 7.4 with tris-buffer and 1 M HCl at 37 °C. Each composite was soaked in SBF for 7 days and the composite was washed with bi-distilled water and acetone, dried, then kept for analysis. The composites surface after soaking were examined via Fourier reflection infrared spectroscopy (FT-IRRS) and scanned by electron microscopy (SEM) to confirm the formation of apatite layer. The morphology (SEM) of the prepared samples was examined with a JXA 840 A Electron Probe Microanalyzer (JEOL, Japan). The scanning electron microscope measurement for each sample was carried out at different magnification.

3. Results

3.1. Phase analysis

XRD patterns of all composites (C) fired at 1250 °C are shown in Fig. 1. The main phase of all composites at 1250 °C is HA having diffraction peaks *d*-spacings equal 3.45, 3.18, 3.09, 2.82, 2.79, 2.73, 2.26, 2.26, 1.94 and 1.84 Å (Card No. 24.33.1998), along with minor phase of $CaO{\cdot}2Al_2O_3$ (CA_2) at *d*-spacing = 4.44, 3.52, 2.76, 2.15 and 1.68 Å [15]. The XRD patterns of all composites fired at 1350 °C are shown in Fig. 2. There is no change in the structures of all composites compared to those the fired at 1250 °C except a little shift of the characteristic peaks of HA to higher 2θ values indicating some interaction and increasing intensity of CA peaks with the content of CA at the expense of HA.

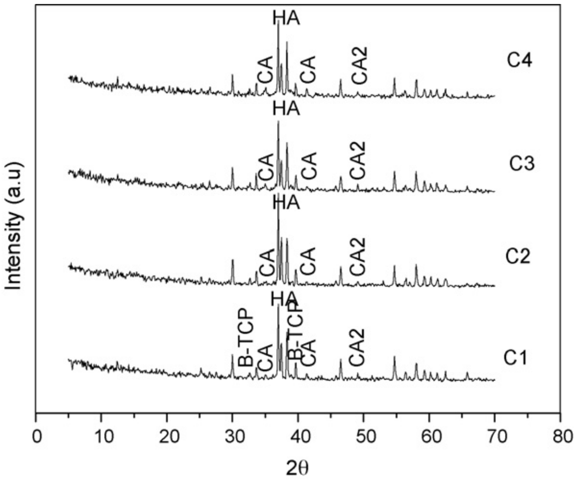

Fig. 1. The XRD patterns of HA/CA composites (C) fired at 1250 °C.

3.2. The FT-IR spectra

FT-IR spectra show absorption bands according to the chemical composition of the composites fired at 1250 °C (Fig. 3). The broadening of the absorption bands is due to O–H stretching at 3567 cm^{-1} and the appearance of O–H liberation mode characterizing HA structure as small shoulder at 635 cm^{-1} for C1 and C2 composites. This band has slight shift to 637 cm^{-1} for C3 and C4 composites reflecting the effect of increasing of CA content. The CA band appears at 839 cm^{-1} in the all composites and increases its intensity with content of CA, where the FT-IR spectra of CA are between 500 and 1000 cm^{-1} [23]. The FT-IR spectra of composites fired at 1350 °C are shown in Fig. 4. The bands of bending mode vibration of OH at 635 cm^{-1} disappeared for the spectra of composites C1, C2 and C3 fired at 1350 °C only, but the bands of stretching mode vibration of OH at 3575 cm^{-1} became more broad, denoting the transformation of hydroxyapatite to oxyapatite structure [24–26]. The phosphate bands are still present in all composites and CA bands also appeared in all composites and their OD increased with content of CA at the expense of HA bands.

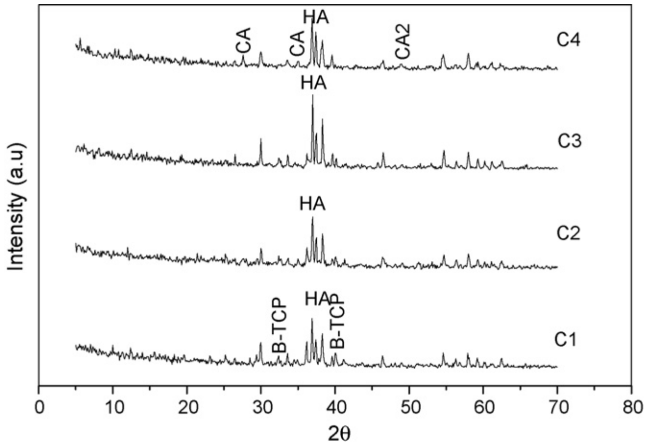

Fig. 2. The XRD patterns of HA/CA composites (C) fired at 1350 °C.

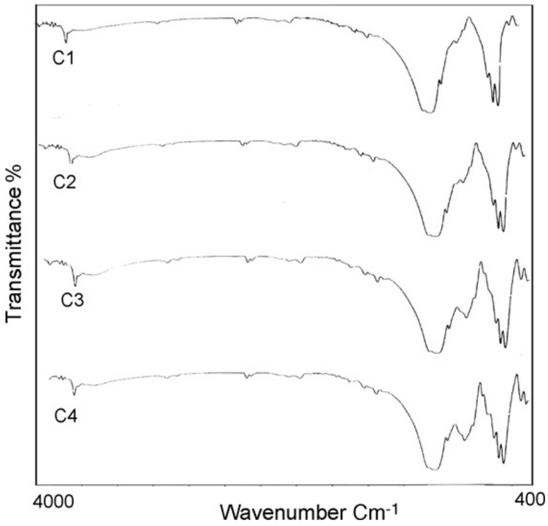

Fig. 3. The FT-IR spectra of HA/CA composites (C) fired at 1250 °C.

3.3. The mechanical properties

The increase in the firing temperature from 1250 to 1350 °C increased the values of H_v and CCS of all the investigated composites (Table 2). Also, the CCS results for the composites fired at both temperatures were gradually enhanced with the increase of CA content in the composite especially at 1350 °C.

3.4. The bioactivity behavior

This study provides information for understanding the fundamental aspects of interaction between bone and biomaterials. The solid composites were investigated post-immersion 7 days after their withdrawal from SBF by FT-IRRS and SEM to confirm the formation of apatite layer onto the composite surface.

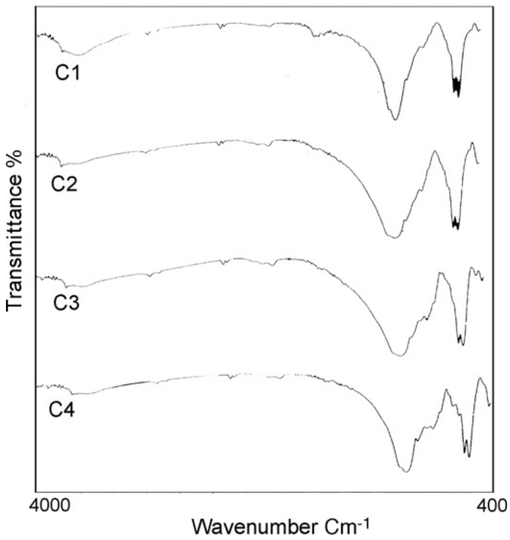

Fig. 4. The FT-IR spectra of HA/CA composites fired at 1350 °C.

Table 2
The mechanical properties of HA/CA composites

	1250 °C[a]				1350 °C[a]			
	C1[b]	C2[b]	C3[b]	C4[b]	C1[b]	C2[b]	C3[b]	C4[b]
Hardness (H_v)	128	132	110	120	175	175	148	140
CCS (MPa)	316	314	327	330	485	494	510	519

[a] Temperature (°C).
[b] Composites.

3.4.1. FT-IRRS assessment

The results of FT-IRRS show that the composites reveal different behavior of these materials when they are in contact with physiological fluids (SBF) for 7 days. The spectra of all composites fired at 1250 and 1350 °C post-immersion are shown in (Figs. 5 and 6). The characteristic groups of bone like apatite such as structural OH, carbonate and/or absorbed water, stretching PO_4^{3-}, liberational OH, bending PO_4^{3-} are cleared at 3570, 1550–1640, 1168–960, 630, 602–558 cm^{-1}, respectively in all composites fired at 1250 and 1350 °C and their intensity are enhanced with increase of CA content as in C3 and C4 composites in both firing temperatures.

3.4.2. SEM morphology

SEM of the composites treated at 1250 and 1350 °C post-immersion in SBF for 7 days at 37 °C is shown in Figs. 7 and 8, respectively. A layer consisting of spongy structure is formed on the whole surface for all composites fired 1250 °C at especially C4 composite characterizing large shape of apatite layer proving effect of CA for the enhancement of apatite formation (Fig. 7). SEM micrograph shows the appearance of large spherical shapes containing a minute pores accumulated to each other which benefit bone in growth as in C4 composite compared to other composites which fired 1350 °C (Fig. 8).

4. Discussion

There is a little transformation of HA to β-TCP in C1 and C2 composites fired at 1250 °C, which was recorded at d-spacing of 2.88, 2.63 and 3.19 Å characterizing β-TCP structure (Card No. 70-2065). The CA phases, CA and $C_{12}A_7$ in the composites C_3 and C_4 fired at 1250 °C were also observed (Fig. 1). These phases prevent the conversion of HA to β-TCP in the composites. The CA phase was recorded at 2.98, 2.95, 2.53, 2.50, 2.20, 2.19, 2.42, 2.39, 1.92 Å while $C_{12}A_7$ phase was recorded at 4.89, 2.99, 2.68 and 2.45 Å according to Scrivener and Campas [19]. From Fig. 2, all peaks intensity of HA increased gradually with increase of CA content till 15% as in C3 composite, after that it decreased till 20% as in C4 composite reflecting the effect of CA content on HA structure. Also, it is notified that all the peaks of composites were broad and sharper for the composites fired at 1250 °C than those fired at 1350 °C proving effect of firing temperature. From these results, it is clear that the increase of CA content prevents the transformation of HA to β-TCP compound in spite of the increase of firing temperature up to 1350 °C, whereas HA is normally transformed to β-TCP at 1170 °C [20].

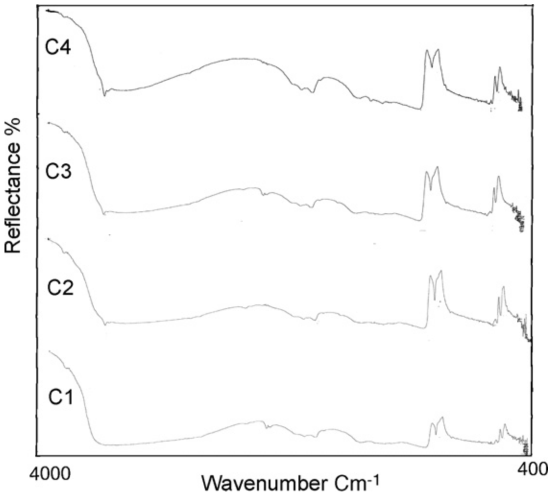

Fig. 5. The FT-IRRS spectra of HA/CA composites treated at 1250 °C post-immersion for 7 days in SBF.

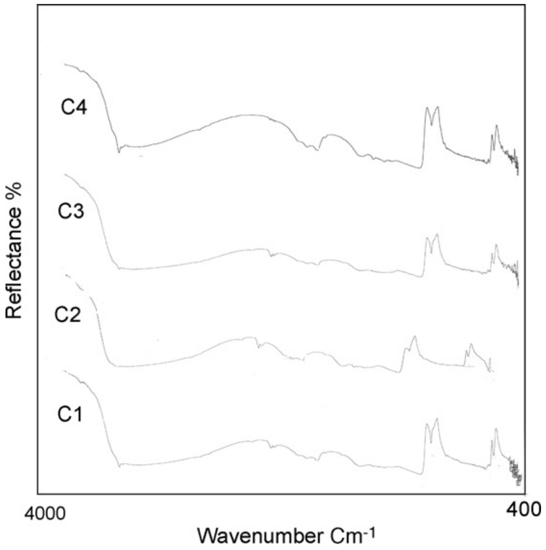

Fig. 6. The FT-IRRS spectra of HA/CA composites treated at 1350 °C post-immersion for 7 days in SBF.

Fig. 3 shows that the increasing intensity of absorption bands between 1623 and 1655 cm^{-1} in C3 and C4 composites could be attributed to the adsorbed H–O–H groups [21] denoting the increase of CA characterizing high affinity to absorbed water in both composites. The improvement of resolution of the PO_4^{-3} absorption bands at 1041 and 569–603 cm^{-1} as well as a shoulder band at 960 cm^{-1} for all composites especially C1and C2 composites [22] were observed. The spectra of C3 and C4 composites exhibited characteristic of HA structure along with CA_2 at 843 cm^{-1} and CA at 750 cm^{-1}. There is a small shoulder at 700 and 960 cm^{-1} of Al–O for $C_{12}A_7$ phase. The crystalline phases of C3 and C4 composites have common spectral features, but an exhibit finger print that uniquely identified $C_{12}A_7$ which has spectrum attributing to a cubic unit cell with polyhedral at lattice positions beside the CA and CA_2 phases [23]. There is some shift to smaller wave number of the latter bands; it may be due to effects of optical change with addition of more ratio of CA to HA into the composites. Fig. 4

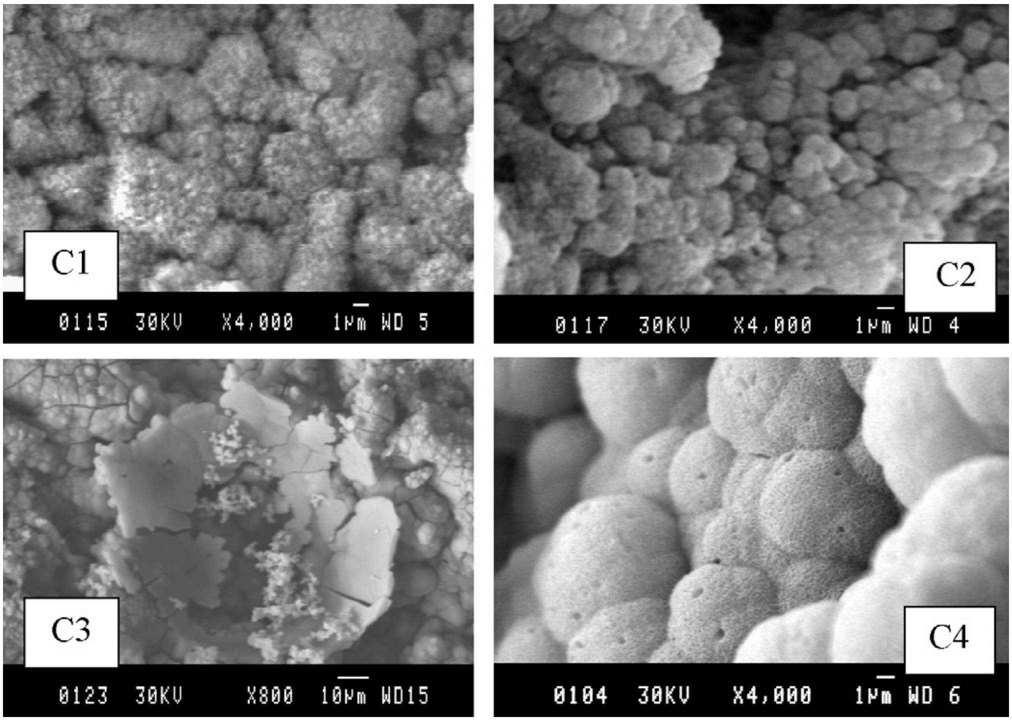

Fig. 7. SEM of HA/CA composites treated at 1250 °C post-immersion for 7 days in SBF.

shows the characteristic bands of HA especially OH and phosphate groups as well as CA bands had lower OD for the composites fired at higher temperature compared to those fired at lower temperature denoting the increase of the firing temperatures affected HA structure in the presence of any content of CA (Figs. 3 and 4). Therefore, FT-IR data coincided with XRD results and confirmed the effect of CA and firing temperature on HA/CA composite structures (Fig. 4).

The H_v and CCS data indicate that high content of CA and firing temperatures improved the mechanical properties of the

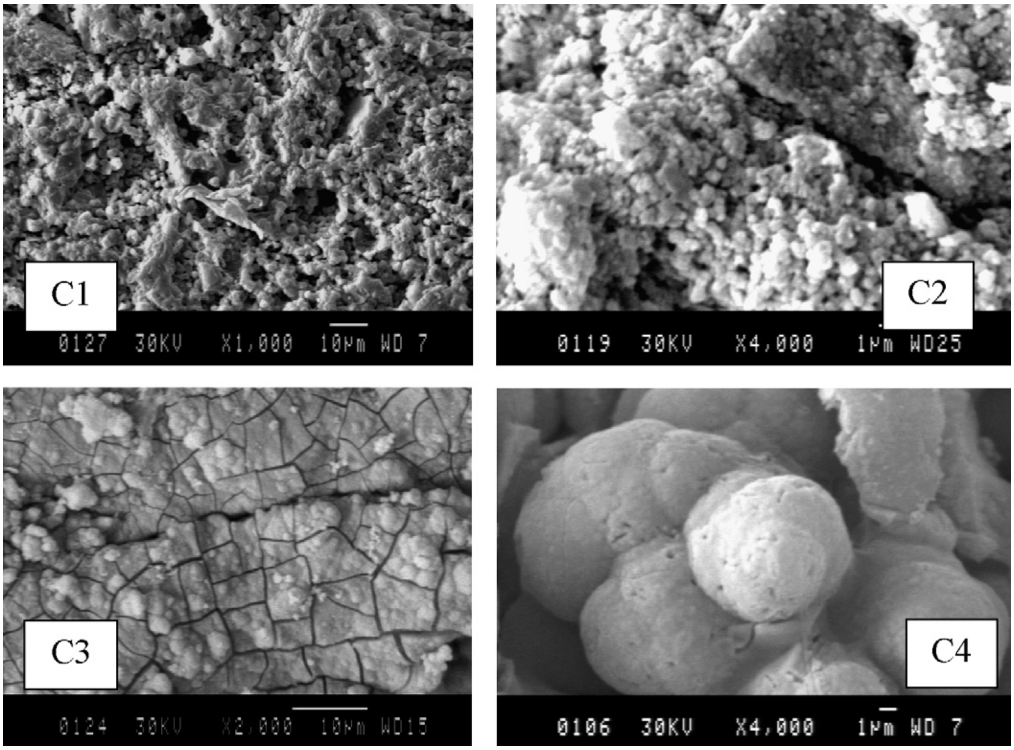

Fig. 8. SEM of HA/CA composites treated at 1350 °C post-immersion for 7 days in SBF.

composites especially in the case of the C3 and C4 composites fired at 1350 °C containing 15 and 20% CA, respectively, especially for CCS test. These results have been attributed to incorporation of a second phase in ceramic matrix [10].

The in vitro test prove that the lower concentration of CA in the composite C1 and C2 indicates slight chemical bioactivity in SBF while the higher concentration of CA in the composites C3 and C4 exhibits high chemical bioactivity post-immersion, which are revealed by FT-IRRS (Figs. 5 and 6). These results indicate that addition of amounts of CA to HA can be effective to significantly and accelerate their bioactivity especially in the composites containing high amount of CA. The bioactive mechanism of composites is rapidly enhanced, and a positive response can be obtained. It is notified that the intensity of these bands were enhanced for the composites treated at 1250 °C compared to these composites fired at 1350 °C. Additionally, the results confirm an important role of CA content in HA/CA composites and firing temperature to accelerate the formation of apatite layer on the composite surface as well as its enhancement as in C4 composite treated at 1250 and 1350 °C containing high content of CA (20%). It was reported that, when most of bioactive materials are soaked in simulated body fluid (SBF), a new calcium phosphate phase that is similar to calcium-deficient apatite crystal is formed in the living body (bone-like apatite layer) on their surfaces. This phenomenon is known as biomimetic formation of apatite layer forming new materials which can provide information on their chemical properties in development of biomaterials [26]. Therefore, the depositions were found using FT-IR containing carbonate ion which plays a vital role in the bone metabolism and they occupy about 3–8 wt.% of the calcified tissue [27]. These depositions were formed as bone-like apatite crystals containing carbonate ions inducing carbonated apatite layer on the surface of composite at 7 days of soaking.

For the SEM observation, the amorphous calcium phosphate film is crystallized by incorporation of hydroxyl, phosphate and carbonate ions from the solution (SBF) to form a bone-like apatite layer. For the composites treated at 1250 °C, SEM shows the presence of irregular shapes on the surface as in C1 composite and many spherical shapes as in C2 composite proving the formation of apatite layer. The size of these spheres are more enhanced and accumulated on each other as in C4 composite as well as they contain minute pores due to the effect of immersion (Fig. 7). This result proves that the increase of CA content into HA/CA composite enhanced the size of spherical shape for apatite layer compared to other composites. The results from Figs. 7 and 8 confirms that the HA/CA composites treated at both firing temperatures have high ability to form an apatite layer on their surfaces, especially C4 composite containing high CA content (20 wt.%) to accelerate apatite formation. This result confirmed the vital role of CA content in the formation of the apatite layer for HA/CA composites that treated at 1250 and 1350 °C. Therefore, the SEM and FT-IRRS results confirmed that C4 composite containing the highest content of CA (20%) at both temperatures had the highest bioactivity and ability to form the carbonated apatite layer compared to other composites.

5. Conclusions

The results of XRD and FT-IR indicated that the presence of any content of CA till 20% in HA/CA composite reduced the transformation of HA to β-TCP at both firing temperatures. It is clear that the increase of CA content prevents the transformation of HA to β-TCP compound. The mechanical properties enhanced with the rise in firing temperature for all composites especially those composites containing high CA content revealing the incorporation of a second phase. The bioactivity study confirmed that C3 composite containing 15% of CA and C4 composite containing 20% of CA treated at 1350 and 1250 °C, respectively, had high ability to form a layer of apatite on the surface. The results prove an important role of both CA content and firing temperature to accelerate the formation of apatite layer on the composite surface. Therefore, the HA/CA composites containing 20 wt.% of CA as in C4 composite could be studied in vivo study in the future for using it as bone substitutes, especially in load bearing sites.

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