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IN VITRO HYDROXYAPATITE FORMING ABILITY OF DICALCIUM SILICATE PREPARED FROM LOCAL RAW MATERIALS AND SINTERED AT 1050 °C

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In this study, Ca_2SiO_4 powders were synthesised using solid state reaction. The starting powders were sintered at 1050 °C for 2 h. Moreover, the in vitro bioactivity of β-Ca_2SiO_4 was investigated by soaking the powders in a simulated body fluid (SBF) for various time periods to analyse the growth of hydroxyapatite (HA) on the surface of these powders. The synthesised powders were characterised by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR), and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis. The simulated body fluid results showed that the β-Ca_2SiO_4 powders had good bioactivity to induce hydroxyapatite formation on their surface. The results obtained showed that (HCA) can be formed on the surface of β-Ca_2SiO_4 soaked in the SBF solution for 1 day, and a continuous layer of dense HCA deposits covered the surface of β-Ca_2SiO_4 powders after 3 days of soaking in the SBF solution. Finally, the results obtained suggest that β-Ca_2SiO_4 ceramics are promising candidates for bone regeneration.

INTRODUCTION

An increasing number of bone defects caused by trauma or inflammation has fueled the huge demand for bone grafting materials for use in orthopaedic surgery [1, 2]. Bioactive materials can bring out a particular biological response at the interface between the tissue and the materials which results in the formation of a bond between the tissues and the materials. The formation of a biologically carbonate-containing hydroxyapatite layer (chemically and crystallographically equivalent to the mineral phase in a bone) on an implant is due to the ion-exchange reaction between the implant and the surrounding body fluids (SBFs) which promote the connection between the natural tissues and the material [3].

Since the discovery of bioglass and bioceramics by Hench and co-workers in 1970 [4], various types of and glass-ceramics, such as bioactive glasses [5-8], and apatite/wollastonite glass ceramics [9-13], have been viewed as bioactive biomaterials for tissue repair and replacement [14-16].

Calcium phosphate cements (CPCs), due to their good self-setting and biocompatibility, have generally been used as clinical bone implants [17]. Indeed, a previous study has shown that their degradation tends to be slow [18].

Recently, various studies have shown that silicon containing biomaterials show good bioactivity and have the potential to enhance osteo-genesis [19-23]. Silicate has shown its excellence in osseous tissue repair in in vitro and in vivo tests [18, 24-28]. Furthermore, Ohtsuki et al [29], showed that calcium ions dissolved from CaO in SiO2-based glasses increase the degree of saturation of the simulated body fluid (SBF) and the hydrated silica formed on their surface provides favourable sites for apatite nucleation. Moreover, Li et al [30], applied a 'charged surface' theory for the explanation of the reaction of bioactive glass in a simulated body fluid and indicated that precipitation of apatite on the surface of these bioactive glass in the SBF is due to the formation of an electric double layer between the bioactive glass and the solution. Therefore, some ceramics, such as dicalcium silicate (Ca_2SiO_4), are regarded as biomaterials

and they may be potential candidates for artificial bone replacement [31].

Furthermore, there are many Ca_2SiO_4 preparation process from SiO_2 and CaO oxides, such as the sol-gel method. However, because of their complicated sample preparation, another method is proposed, which is based on replacing expensive materials by other less expensive raw materials which are abundantly available in the world. Many works have been published with regards to the valorisation of native raw materials within bioceramic applications [32-36].

Consequently, the main goal of this study is to evaluate the bioactivity of pure β-Ca_2SiO_4 (prepared from local raw materials) by examining the hydroxyapatite (HA) formation on their surface in an SBF solution.

EXPERIMENTAL

Preparation of powders

Ca_2SiO_4 powders were synthesised through a solid -state reaction method according to our previous study [34]. A mixture of high purities, SiO_2 and CaO, extracted from local $CaCO_3$ ($CaCO_3$ was calcined at 900 °C for 9 hours (h) then hydrated by distilled water and then followed by a second calcination at 800 °C for 2 h). The purity of the obtained CaO was about 99.7 %, using fluorescence XRD analysis. As reported by Ming et al [37], this raw material has a high-purity calcium oxide (CaO ≥ 99.0 %).

CaO and SiO_2 with a CaO/SiO_2 molar ratio equal to two were mixed using wet milling for 3 h, which was then calcined at 650 °C for 2 hours. Subsequently, the powder mixtures were sintered at 1050 °C for 2 hours.

In vitro tests (apatite forming ability)

The in vitro properties of the sintered specimens were investigated by their apatite forming ability in the simulated body fluid (SBF). The powders sintered at 1050 °C for 2 h were soaked in an SBF solution whose ion concentration was nearly equal to those of human body blood plasma, as shown in Table 1 [38]. The SBF solution was buffered at pH = 7.4 with trimethanol aminomethane-HCl-6N. The powders were immersed in the SBF solution at 37 °C for 4 hours and for 1, 3, 7, 15 days. After soaking for various periods, the samples were removed from the fluids with deionised water, and dried at room temperature.

Characterisations

The phase identification and morphology of the powders before and after soaking in the SBF solutions were characterised by X-ray diffraction (XRD) (BRUKER, D8 ADVANCE) (Karlsruhe, Germany) with CuKα radiation (λ = 0.154 nm) and an Ni filter, with a working voltage of 40 kV and a working current of 30 mA, Fourier-transform infrared (FTIR) spectroscopy (Bruker Equinox 55: 4000-400 cm^{-1}). The surfaces of the powders were observed by Scanning Electron Microscopy (SEM) (HITACHI, JSM-6301 F) (Tokyo, Japan) working at 7 kV as the accelerating voltage and by Energy Dispersive Spectrometry (EDS) (JEOL JSM-6400). The changes in the concentrations of the Ca, P and Si ions in the SBF solution after soaking were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Spectro Ciros Vision, Karlsruhe, Germany).

RESULTS AND DISCUSSION

Characterisation of the β-Ca_2SiO_4 powders

Figure 1 (0 hours) shows the XRD patterns of the dicalcium silicate powders sintered at 1050 °C for 2 h before soaking in the SBF solution. The XRD spectrum demonstrates the formation of the β-Ca_2SiO_4 phase (JCPDS card number 72-1130). The peak positions are in good agreement with those of reference [39].

The FTIR spectra of the dicalcium silicate powders sintered at 1050 °C and before soaking in the SBF are illustrated in Figure 2 (0 hour). It is obvious that the spectrum of the dicalcium silicate powder was dominated by the Si-O vibrations. The peaks at 472, and 902 cm^{-1} are due to the Si-O vibration and the peaks mainly occur in the range of 519 and 994 cm^{-1} are due to the O-Si-O vibration [40, 41]. Moreover, an OH^{-1} absorption band around a 3500 cm^{-1} can be seen in this spectrum.

Apatite-formation ability of the β-Ca_2SiO_4 powders

Figure 1 shows the XRD patterns of the β-Ca_2SiO_4 powders before and after soaking in the SBF solution for various time periods. It is obvious that the characteristic peak intensities of β-Ca_2SiO_4 decrease with an increase in the soaking time and disappeared after 1 day of soaking. New peaks for $CaCO_3$ were apparent after 1 day (the JCPDS card number for $CaCO_3$ is 88-1807),

Table 1. Ion concentration of the SBF in comparison to human blood plasma.

Types	Ion concentrations [mM]							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO^{3-}	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

while the characteristic peaks of HA in 2θ = 32 and 26 appeared after 1 day of soaking, indicating the formation.

The FTIR spectra of the powders sintered at 1050 °C before and after soaking in the SBF are displayed in Figure 2. The IR spectrum of the β-Ca_2SiO_4 powders is also given in this figure, showing the spectral characteristics of β-Ca_2SiO_4. When the soaking time increased, the intensity of the silicate absorption bands decreased. Simultaneously, new absorption bands at 566 and 603 cm^{-1} and a broad band appeared after 1 day of soaking, and these bands were split from the P–O bending vibration (υ4) of the PO_4^{3-} group around 598 cm^{-1}. The band around 1100 cm^{-1} could be attributed to the P–O stretching vibration (υ3) mode [42]. According to the report of IR correlation charts [43], all these bands were characteristic of HA crystals, which indicated the formation of HA on the surface of the β-Ca_2SiO_4 powders. A broad OH^- absorption band around 3500 cm^{-1} can be seen in these spectra. Furthermore, the C-O stretching of the CO_3^{2-} groups at 872, 1419 and 1490 cm^{-1} were observed after 1 day of soaking, which indicated that HCA was formed on the surface of the β-Ca_2SiO_4 powders after soaking in the SBF solution. These results further confirmed that a carbonate-containing hydroxyapatite layer could be induced to deposit on the surface of β-Ca_2SiO_4 ceramics in the SBF of HA on the surface of the β-Ca_2SiO_4 powders.

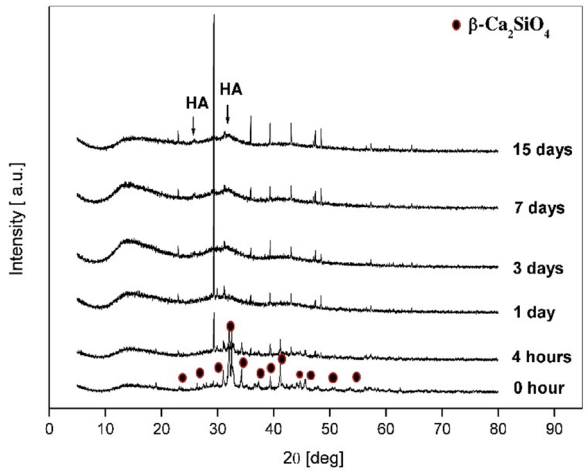

Figure 1. XRD patterns of the β-Ca_2SiO_4 powders sintered at 1050 °C for 2 hours before and after soaking in the SBF solution for various periods.

The surface morphology of the β-Ca_2SiO_4 powders before and after soaking in the SBF solution for 4 hours, and 1, 3, 7 and 15 days are presented in Figure 3 and Table 2. In comparison with the particles before soaking in the SBF solution presented in Figure 3a. small ball-like particles appeared on the surface of the β-Ca_2SiO_4 powders soaked in the SBF for 4 h as shown Figure 3b. Up to 1 day of soaking, ball-like particles were observed

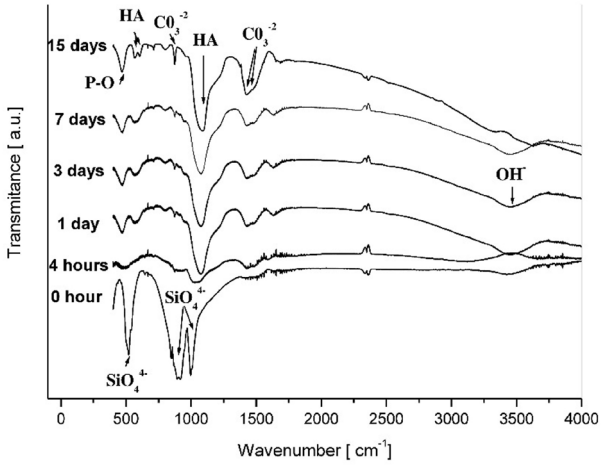

Figure 2. FTIR spectra of the β-Ca_2SiO_4 powders sintered at 1050 °C for 2 h before and after soaking in the SBF solution for various periods.

inside the pores and on the surface of the β-Ca_2SiO_4 powders (Figure 3c). After prolonged soaking for up to 3 days, the surface morphology of the powders changed and a continuous layer of dense deposits formed on the surface of the β-Ca_2SiO_4 powders as given in Figures 3 (d, e, f). The results are in good agreement with previous studies [39].

Table 2. EDS quantitative analysis (element concentration) of the β-Ca_2SiO_4 powders after soaking in the SBF solution for 15 days.

Element	% Mass	% Atomic
Si K	7.43	37.30
P K	4.91	22.39
Ca K	11.45	40.31
Total	23.79	

Table 2 shows the EDS quantitative analysis (elements concentration) of the powders after 15 days of soaking in the SBF solution. The EDS quantitative analysis of the continuous layer formed on the surface of powders soaked in the SBF for 15 days gave a Ca/P ratio of around 1.80 which is slightly higher than that in hydroxyapatite. This fact suggests that HCA is formed on the surface of the β-Ca2SiO4 powders (a Ca/P ratio value greater than the pure hydroxyapatite) according to the results reported by other authors [44-46]. The HCA formation is a consequence of the usually high levels of calcium in the solution near the surface, due to dissolution of β-Ca_2SiO_4, and the enrichment with the carbonate ions present in the SBF that give rise to the HCA on the surface.

The formation mechanism of apatite was proposed by Hench et al [47]. The exchange of calcium ions in ceramics with H^+ in an SBF solution gives rise

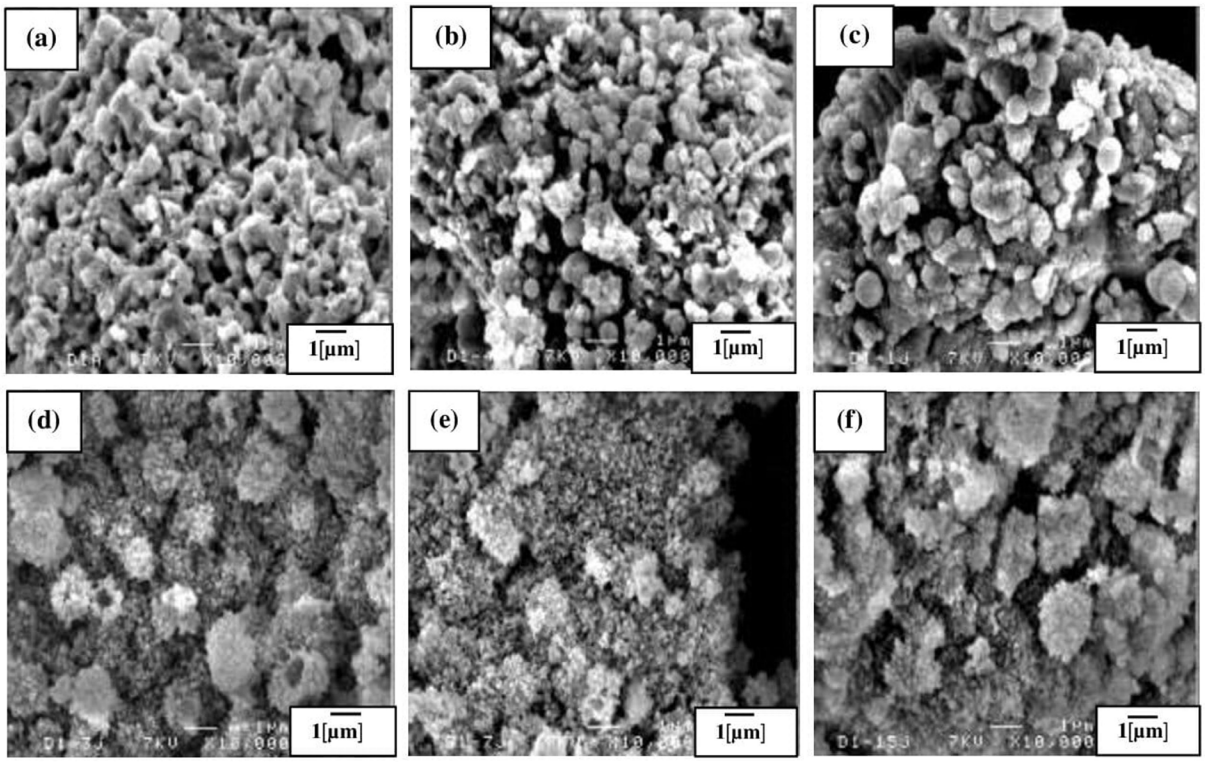

Figure 3. SEM micrographs of the β-Ca_2SiO_4 powders sintered at 1050 °C for 2 h before and after soaking in the SBF solution for various periods; (a) 0 h, (b) 4 h, (c) 1 d, (d) 3 d, (e) 7 d, (f) 15 d.

to the formation of silanol (Si-OH) in the surface layer, an increase in the pH value at the ceramics-SBF interface, and eventually the production of a negatively charged surface with the functional group (Si-O-). This later had been proposed to be a catalysing agent and could provide specific favourable sites for the apatite nucleation [48, 49].

Concentration changes of Ca, P and Si in the SBF solution

Figure 4 shows the changes in the concentrations of Ca, P and Si in the SBF solutions measured by ICP after soaking for various time periods. It is obvious that the ion concentrations of the SBF solutions changed markedly after soaking in the SBF solution. The Ca and Si concentrations in the SBF increased with an increase in the soaking time up to 1 day. Afterwards, the Ca concentration in the SBF decreased after 1 day of soaking. In contrast to the increase in the Ca and Si concentration, the P concentration of the SBF solutions decreased during the soaking periods.

The increases in the calcium and silicon concentrations were attributed to the dissolution of these ions from the β-Ca_2SiO_4 powders. Moreover, the decrease in the calcium ions after 1 day of soaking is due to the faster consumption of Ca ions during the subsequent formation of HA on the surface of the powders. The decrease in the phosphorus concentration was attributed to the formation of both amorphous calcium

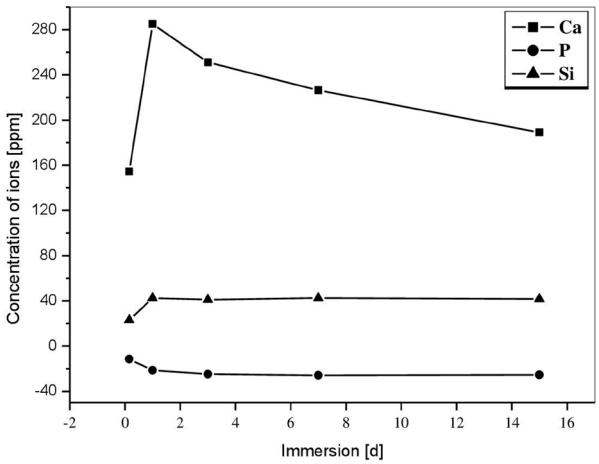

Figure 4. Changes in the Ca, P and Si concentrations of the SBF solutions after soaking the β-Ca_2SiO_4 powders for various periods.

phosphate and crystalline apatite on the surface of the powders by consuming the P ions from the SBF solutions. The ICP measurement results suggest that the Ca and Si concentrations increased while the P concentration decreased due to the formation of HCA on the surface of the β-Ca_2SiO_4 powders during soaking in the SBF solution [50].

CONCLUSIONS

β-Ca_2SiO_4 was fabricated using a solid-state reaction. The results obtained indicated that the β-Ca_2SiO_4 prepared from the local raw materials ($CaCO_3$) have higher reactivity in the SBF solution by showing strong hydration when soaked in it, and the hydration was favourable for formation of HCA on the surface of the powders. Furthermore, HCA was formed on the surface of powders soaked in the SBF solution for 1 day. With longer soaking periods (3, 7 and 15 days), the surface of the powders was covered by a dense and continuous HCA layer. Therefore, β-Ca_2SiO_4 ceramics should have the potential for good bioactivity and could rapidly induce HAp formation after soaking in SBF and, thus, may be used as candidate of bone repair biomaterials.

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