



Bioactive ceramic powder prepared using a new sol-gel process

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Abstract

Bioactive ceramic powder containing 45% SiO₂, 24.5% CaO, 24.5% Na₂O and 6% P₂O₅ (wt.%) was synthesized using a new sol-gel process. The prepared material was investigated by several physico-chemical methods. The X-ray diffraction showed the presence of two crystalline phases, Na₆Ca₃Si₆O₁₈ and NaCaPO₄. The high specific surface area (47 m²/g) and a mesoporous structure with the average pore size of 2.6 nm were confirmed using low-temperature nitrogen adsorption technique. *In vitro* experiments were carried out by soaking the powder sample in simulated body fluid (SBF) at different times. XRD, FTIR and SEM coupled with EDS were used for *in vitro* evaluation of bioactivity. The obtained results confirmed that the ceramic powder can be used as biomaterial for bone tissue engineering due to its high bioactivity expressed by the rapid formation of a biological carbonated hydroxyapatite layer on its surface after *in vitro* assay.

Keywords: bioactive powder, structural characterization, *in vitro* bioactivity, hydroxyapatite

I. Introduction

Bioactive glasses are traditionally processed by melting method, in which precursor materials are melted at high temperature. The obtained liquid is quenched to form an amorphous material at room temperature. The first melt-derived bioglass 45S5 containing 45% SiO₂, 24.5% CaO, 24.5% Na₂O and 6% P₂O₅ (wt.%) was prepared by Hench in 1969 [1–4]. Recently, the sol-gel method has been widely used to prepare bioglasses, due to its higher surface area in comparison to the melt-derived bioglass [5–7]. The high surface area of the sol-gel bioglass enhances the interfacial reactions between the material and physiological environment that accelerates the formation of biological apatite layer. Many bioglasses have been formed by sol-gel method, but a similar glass to the 45S5 one has not been synthesized successfully yet because of its crystallization during sol-gel procedure [8]. Several studies reported the preparation of quaternary SiO₂-CaO-Na₂O-P₂O₅ bioglasses by sol-gel method, but their compositions were very different from the 45S5 bioglass [9–11]. While the 45S5 bioglass prepared by sol-gel process has not been observed yet, an alternative approach has to be considered for synthe-

sis of a similar quaternary system with high bioactivity. Thus, in this study we reported a new sol-gel process to prepare a ceramic powder having the same composition as the bioglass 45S5. This ceramic powder revealed the high bioactivity after *in vitro* assay in SBF solution.

II. Experimental methods

2.1. Materials

Bioactive ceramic powder containing 45% SiO₂, 24.5% CaO, 24.5% Na₂O and 6% P₂O₅ (wt.%) was synthesized by sol-gel method and the following precursor materials were used: tetraethyl orthosilicate (TEOS) (Aldrich, 99%), triethyl phosphate (TEP) (Sigma-Aldrich, 99.8%), calcium nitrate tetrahydrate (Fluka Biochemika, 99%) and sodium nitrate (Sigma-Aldrich, 99%).

2.2. Powder preparation

The bioactive ceramic powder was prepared by mixing 6.242 g of TEOS and 0.616 g of TEP in 15 ml of distilled water and stirring for 2 hours by magnetic agitation at 500 rpm. The pH value of the mixture was adjusted to 1.5 using HNO₃ solution. After that 4.127 g of Ca(NO₃)₂·4H₂O and 2.688 g of NaNO₃ were respectively added to the mixture at 60-minute intervals. Fi-

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nally, 3 drops of 30% ammonia solution were poured into the mixture and continuously stirred for 4 hours to obtain a condensed gel. The resulting gel was frozen and then freeze-dried at -60°C for 24 hours to remove the residual water and ethanol. The obtained dry gel was heated at 1000°C for 2 hours and converted into ceramic powder. The synthesis protocols are summarized in Fig. 1.

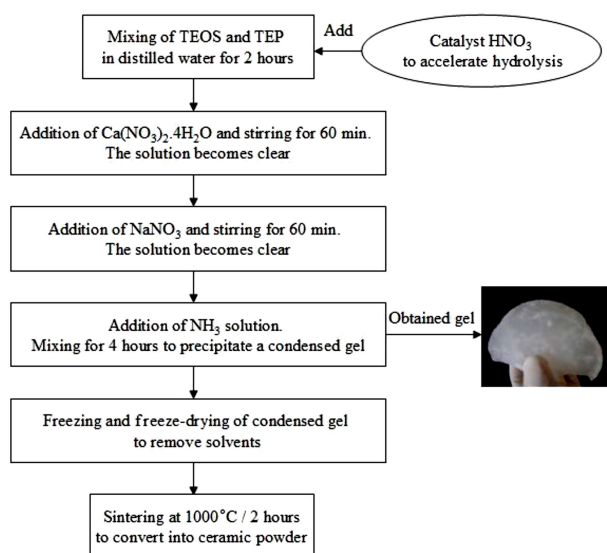


Figure 1. Flowchart of powder synthesis protocol

2.3. *In vitro* bioactive test

In vitro bioactivity of the ceramic powder was investigated by soaking 100 mg of material in 200 ml of SBF solution at body temperature. The SBF composition is similar to that of human blood plasma. It was prepared by dissolving NaCl, NaHCO_3 , KCl, $\text{K}_2\text{HPO}_3 \times 3 \text{H}_2\text{O}$, $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ and CaCl_2 in distilled water and buffered with $(\text{CH}_2\text{OH})_3\text{CNH}_2$ and HCl (6 N) to adjust the pH value at 7.4 according to the Kokubo's method [12].

2.4. Physico-chemical characterizations

The ceramic powders before and after soaking in SBF solution were investigated by using several physico-chemical techniques. The specific surface area and porosity of ceramic powder were determined by the low-temperature nitrogen adsorption-desorption measurements on multi-point BET, ASAP 2010 Analyzer. In order to identify the crystalline phases of synthetic material and evaluate the formation of apatite layer after *in vitro* assay, X-ray diffraction (XRD) measurements were realized on Bruker D8 Advance diffractometer. Powder samples were mixed homogeneously with cyclohexane and dropped on the surfaces of plastic tablets. Then,

these tablets were dried to remove the solvent and introduced into diffractometer. The XRD data were acquired in the range of $10\text{--}70^{\circ}$ (2θ) with a scanning speed of $1^{\circ}/\text{min}$. The Fourier transformed infrared spectroscopy (FTIR) (Bruker Equinox 55) was employed to identify the functional groups of the ceramic powder before and after *in vitro* assay. Powder materials were ground and mixed thoroughly with KBr powder in the ratio 1:100. FTIR spectra were recorded in the wavenumber range of 400 and 4000 cm^{-1} , at a resolution of 2 cm^{-1} . Scanning electron microscopy (SEM) (JEOL JSM 6301) coupled with energy dispersive X-ray spectroscopy (EDS) was used to evaluate the morphological surface of ceramic powder as a function of soaking time in SBF solution and determine the Ca/P elemental ratio in the apatite layer after *in vitro* assay.

III. Results and discussion

3.1. Porosity characterization

The nitrogen adsorption and desorption isotherm curve of the bioactive powder is presented in Fig. 2. It exhibited a type IV character according to the IUPAC classification, typical for a mesoporous material containing the pores between 2 and 50 nm in diameter. The specific surface area, pore volume and pore size of the ceramic powder are listed in Table 1. It can be seen that the specific surface area of the sol-gel derived ceramic powder is much higher than that obtained for the melt-derived glass 45S5 [6].

3.2. Phase composition

Figure 3 shows the XRD diagrams of the ceramic powder before and after soaking in SBF solution. The

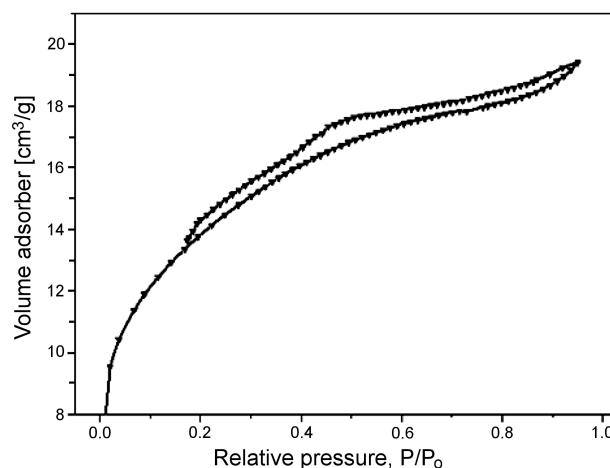


Figure 2. N_2 adsorption-desorption isotherm line of ceramic powder

Table 1. Specific surface area and porosity of ceramic powder

Samples	S_{BET} [m^2/g]	Pore volume [m^3/g]	Median pore diameter [nm]
Bioglass 45S5	0.15–2.7	-	-
Ceramic powder	47.0	0.030	2.6

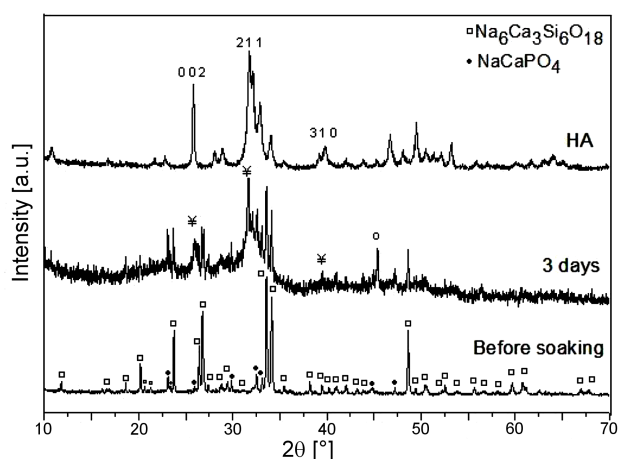


Figure 3. XRD diagrams of ceramic powder before and after soaking in SBF solution

identification of crystalline phases of synthetic ceramic was performed using the literature data [13–15] and the XRD diagram of hydroxyapatite (HA pattern in Fig. 3) was also presented for comparison [16]. The XRD pattern of the annealed ceramic powder exhibited two crystalline phases. The major crystalline phase is $\text{Na}_6\text{Ca}_3\text{Si}_6\text{O}_{18}$, which is close to $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ phase found in the heat treated 45S5 bioactive glass [17,18]. The minor phase is NaCaPO_4 and its existence illustrated the presence of phosphorus ions in the ceramic composition adapting the chemical stoichiometry of the initial synthetic system.

After 3 days of soaking in SBF solution, the characteristic peaks of $\text{Na}_6\text{Ca}_3\text{Si}_6\text{O}_{18}$ phase become weaker or disappear while two new peaks at 26° and 32° (2θ) emerge in the diffractogram of the ceramic powder. These new peaks correspond to the (002) and (211) planes in the structure of hydroxyapatite crystals, respectively. The obtained results confirmed bioactivity of the ceramic powder. The disappearance of $\text{Na}_6\text{Ca}_3\text{Si}_6\text{O}_{18}$ diffraction peaks continues while the intensities of two apatite characteristic peaks are not much modified with an increase in the soaking time from 3 days to 15 days. This result indicated that the apatite formation on the surface of ceramic powder could be completed almost after only 3 days of immersion.

3.3. IR spectroscopy analysis

Figure 4 shows the FTIR spectra of the ceramic powder before and after soaking in SBF solution. The identification of functional bands referred to previous studies [10,17,18]. The IR spectrum of synthetic hydroxyapatite (HA spectra in Fig. 4) was used as a reference to identify the formation of apatite phase on the surface of the ceramic particles as function of soaking time [19]. The FTIR spectrum of the ceramic powder presents the characteristic bands of a phospho-silicate network. Three bands at 1094 , 730 and 690 cm^{-1} are attributed to the symmetric stretching Si–O–Si vibrations. The band at 1035 cm^{-1} is assigned to the asymmetric stretching Si–O–Si vibration. The band at 920 cm^{-1} corresponds to Si–

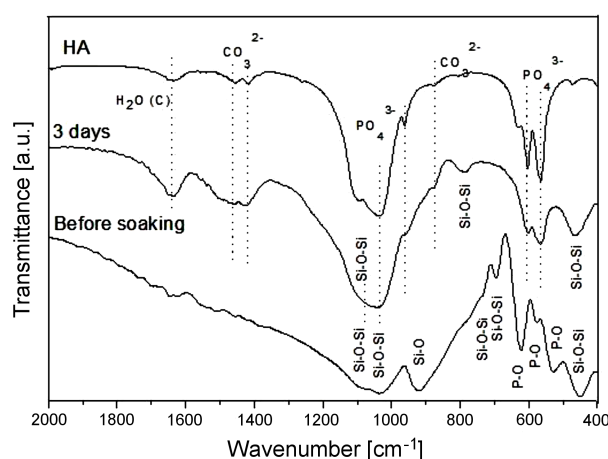


Figure 4. FTIR spectra of ceramic powder before and after soaking in SBF solution

O bond. The band at 450 cm^{-1} is attributed to the angular Si–O–Si deformation vibration. The presence of a sodium calcium phosphate is revealed by three characteristic bands at 620 , 580 and 530 cm^{-1} . They are attributed to P–O bonds in NaCaPO_4 crystalline phase that was identified by X-ray diffraction.

After soaking in SBF solution, the initial structure of the ceramic powder changed significantly due to the interfacial interactions between the material and the physiological solution. Consequently, the spectrum of the ceramic powder contains four new well-defined phosphate bands at 565 , 603 , 960 and 1039 cm^{-1} after 3 days of soaking in SBF solution. They are assigned to the stretching vibrations of PO_4^{3-} groups in apatite crystalline phase. Two carbonate bands at 874 and 1420 cm^{-1} are also observed. The obtained results confirmed the formation of a carbonated hydroxyapatite layer on the surface of the ceramic particles after *in vitro* assay. In addition, the IR spectrum of the ceramic powder soaked in SBF exhibits three new bands of Si–O–Si at 470 cm^{-1} , 799 cm^{-1} (bending vibration) and 1070 cm^{-1} (stretching vibration). They are characteristic for the pure silica [4]. This result confirmed the formation of a SiO_2 gel on the surface of the ceramic particles and proposed interactive mechanism between bioactive glass and physiological solution described by Hench [1–3]. It is summarized in following several steps: (1) rapid exchange of protonated H_3O^+ from the physiological solution with Ca^{2+} , Na^+ ions in bioglass to form the Si–OH groups, (2) loss of soluble silica as $\text{Si}(\text{OH})_4$ by breaking Si–O–Si bridging links and subsequent formation of silanol groups at the surface in this process, (3) condensation and repolymerization of surface silanols to form SiO_2 -rich surface layer, (4) migration of Ca^{2+} and PO_4^{3-} from bioglass through the surface of the silica-rich layer and deposition of these ions from SBF solution to form an amorphous calcium phosphate (ACP) film on top of the SiO_2 -rich layer, (5) incorporation of OH^- , CO_3^{2-} from the solution and subsequent crystallization of the ACP layer to form a hydroxycarbonate apatite layer (HCA).

After 15 days of soaking in SBF solution, the intensities of phosphate characteristic bands at 565, 603, 960 and 1039 cm^{-1} did not change much. This result is fully in accordance with the XRD analyses, in which the characteristic peaks of hydroxyapatite did not change much from 3 to 15 days of immersion. The apatite layer could be formed completely on the surface of the ceramic particles after 3 days of soaking in SBF solution. On the other hand, the characteristic bands of silica gel become more intensive after 15 days of immersion. This confirmed the growth of silica layer as a function of the

soaking time in SBF liquid.

3.4. SEM and EDS analysis

Figure 5 shows SEM images of the ceramic powder before and after soaking in SBF solution. The surface of the ceramic particles before soaking was smooth. After immersion in SBF solution, it was modified strongly due to the interfacial chemical reactions between the ceramic powder and the physiological solution. After 1 day of immersion, the small particles began to form, but the surface of the ceramic particles was not much mod-

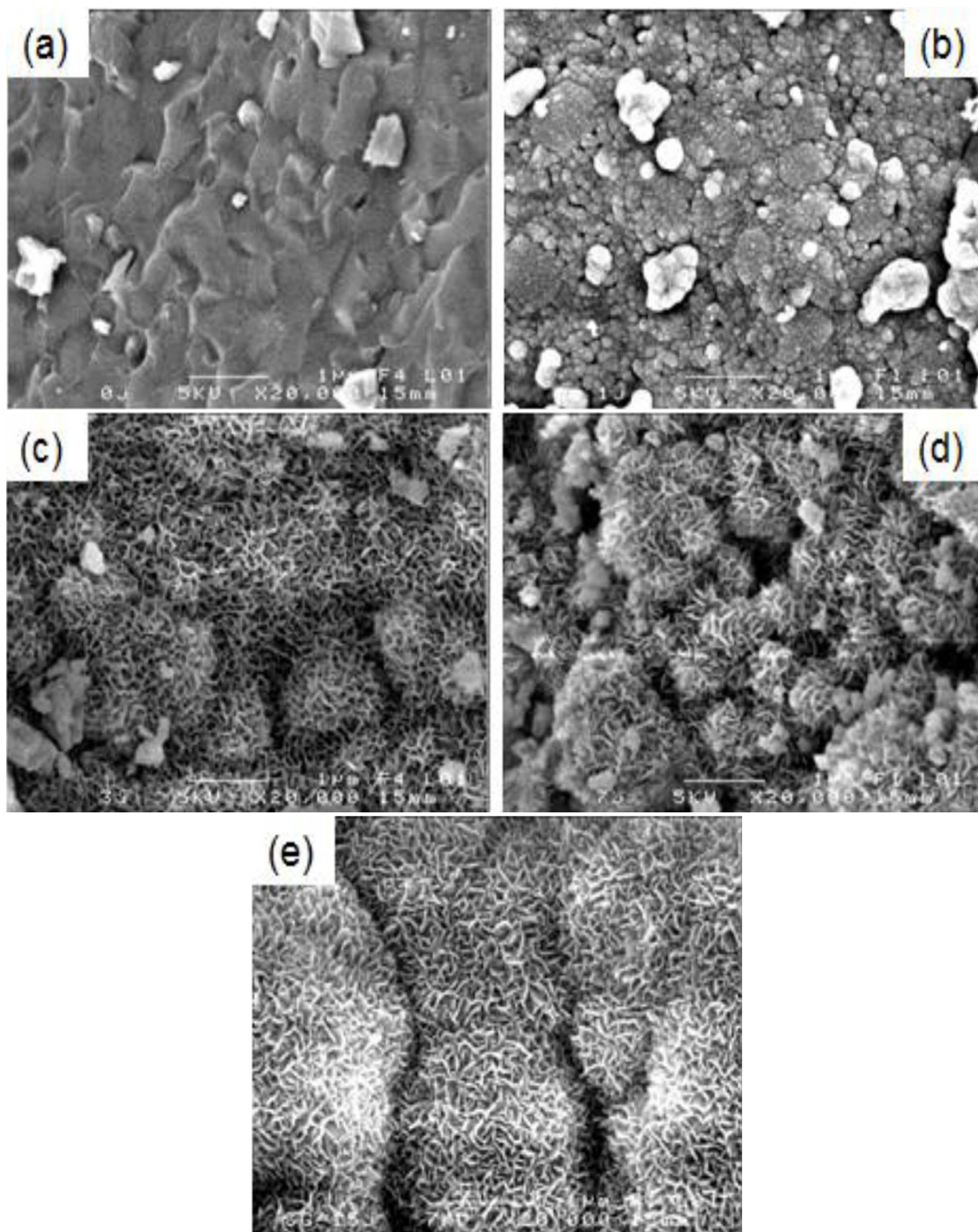


Figure 5. SEM images of ceramic powder: a) initial ceramic powder, b) after 1 day, c) after 3 days, d) after 7 days and e) after 15 days of soaking in SBF solution

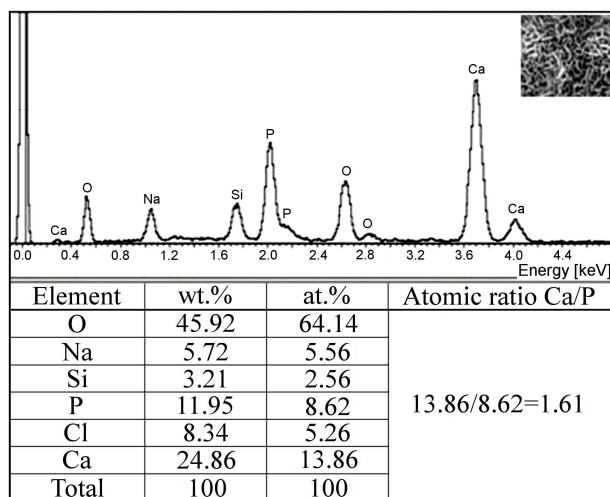


Figure 6. EDS spectrum of ceramic powder after 15 days of soaking in SBF solution

ified. After 3 days of immersion, the surface of the ceramic particles was covered completely with a homogeneous layer with apatite crystalline structure (according to the XRD and FTIR results). After 15 days of immersion, the surface of the ceramic particles changes only a little bit more, compared to that obtained after 3 days soaking. The above results confirmed the high bioactivity of the synthetic ceramic by formation of a dense and visible apatite layer after only 3 days in SBF solution. In previous studies [20,21], a series of B_2O_3 -CaO-SiO₂-P₂O₅ showed their bioactivity through the creation of apatite particles after 3 days and a complete apatite layer formation after 7 days of immersion in SBF fluid.

The EDS spectrum of the ceramic powder after 15 days of immersion in SBF solution is shown in Fig. 6. It shows the presence of Na, Si, P, Cl, Ca and O elements. The obtained Ca/P ratio was of 1.61. It was nearly equal to the chemical stoichiometry of hydroxyapatite (Ca/P = 1.67). This result confirmed also the formation of bone-like apatite on the surface of the ceramic particles.

IV. Conclusions

Bioactive ceramic powder containing 45% SiO₂, 24.5% CaO, 24.5% Na₂O and 6% P₂O₅ (wt.%) has been successfully prepared using a new sol-gel process. It consisted of two crystalline phases: Na₆Ca₃Si₆O₁₈ and NaCaPO₄ and has a high specific surface area. The *in vitro* assay was carried out by soaking ceramic samples in SBF solution at different times. The obtained results confirmed the bioactivity of this ceramic powder by the apatite formation within a few days.

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