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Microstructural characterization and in vitro apatite formation in $CaO–P_2O_5–TiO_2–MgO–Na_2O$ glass-ceramics

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Abstract

Bulk calcium phosphate glass and glass–ceramic with CaO/P_2O_5 molar ratio = 1.25 were prepared through introducing small amounts of Na_2O, MgO, and TiO_2 into the glass. Using XRD analysis, biocompatible β-$Ca_2P_2O_7$ (β-DCP) and more soluble Ca and Na-containing phases were clearly identified in the microstructure. In vitro bioactivity was assessed by immersion testing in SBF-simulated body fluid and apatite crystals were formed after 4 and 8 weeks of soaking. Porous microstructure was produced through dissolution of soluble phases and desirable porous structure may be achieved by controlling size and distribution of these phases and the leaching process. Therefore, this glass–ceramic is expected to be used as bone repair and regeneration implants and drug delivery carrier.

Keywords: Apatite; Bioactivity; CaO/P_2O_5; Glasses; Crystallization; Glass ceramics; Microstructure–final

1. Introduction

Different kinds of silicate-based bioactive glasses [1,2] and glass–ceramics [3,4] have been developed because glass-based materials may be prepared in desirable compositions and structurally designed by controlling phases and the size of crystals precipitated in the glasses. It is also well accepted that silica-free calcium phosphate glasses and glass–ceramics have a high potential to be used as biomaterials because their chemical composition is close to that of natural bone. Preparation of glass–ceramics with high CaO/P_2O_5 ratio, containing large amounts of calcium phosphate crystals, is believed to be one of the best approaches to obtain bioceramic implants suitable for bone replacement/regeneration. These implants may also act as drug carriers due to their ability to partially degrade in physiological media and let drug release to occur. However, it is not easy to prepare calcium phosphate glasses with the composition of a high CaO/P_2O_5 ratio (molar ratio >1.2) in the pyrophosphate region,[5] since very high temperatures are required and crystallization tends to occur. Abe et al., prepared porous glass–ceramics with $CaO/P_2O_5 = 1.5$ but containing large amounts of TiO_2, 25 mol%.[6] Kasuga et al. reported the preparation of phosphate glasses in the pyrophosphate region ($CaO/P_2O_5 \approx 2$) by introducing 10 mol% total of Na_2O and TiO_2, but the thickness of the resulting glasses was below 1 mm and the glasses are mainly used for further powder sintering.[7,8]

Furthermore, in vitro studies showed that, when immersed in simulated body fluid (SBF), certain compositions of silicate-based bioactive glasses and glass-ceramics such as 45S5 bioglass® and A/W glass–ceramic form an apatite layer on the surfaces,[9,10] which is chemically and structurally equivalent to the mineral phase in bone. The apatite formation on the surfaces of P_2O_5-free glasses and glass–ceramics in SBF solution were also observed.[11,12] However, no apatite layer formed on $CaO–P_2O_5$ glasses under the same condition.[13] In this work, bulk calcium phosphate glass and glass–ceramic with CaO/P_2O_5 molar ratio = 1.25 were prepared through introducing a small content of Na_2O, MgO and TiO_2 into the glass. Microstructural characterization was performed by X-ray diffraction (XRD) and scanning electron microscopy (SEM) analysis. In vitro apatite layer formation ability was assessed by

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soaking glass–ceramic samples in SBF solution up to 8 weeks and apatite layer analyzed by SEM and X-ray photoelectron microscopy (XPS).

2. Experimental procedure

2.1. Sample preparation

The composition of the glass in molar percentage is given in Table 1. The mixture of starting materials, which were reagent grade $CaCO_3$, $Ca(H_2PO_4)_2$, $Mg(CH_3COO)_2$, Na_2CO_3 and TiO_2 (Sigma-Aldrich, USA), was placed with water in a Pyrex beaker and stirred to make a homogeneous slurry. The slurry was dried at ~200°C for 12 h. The resulting dried product was pulverized into fine powders and used as the batch. Using a heating rate of 10°C min^{-1}, the batch was melted in a platinum crucible at 1400°C for 1 h under an ambient atmosphere, then quickly poured into a stainless steel mold preheated at 300°C, resulting in the formation of bulk glasses without cracks. Once removed from the mold, the as-obtained glass was annealed at 620°C for 30 min and naturally cooled to room temperature inside the furnace. Volume crystallization was achieved through an efficient two-step heat treatment as reported by Hosono et al.,[14] according to DTA results in Fig. 1. In step 1 (nucleation), specimens were heated at a rate of approximately 300°C/h from room temperature to 690°C and held at this temperature for 24 h. Then in step 2 (crystal growth), the samples were heated at approximately 180°C/h from 690 to 790°C and held at the latter temperature for 48 h.

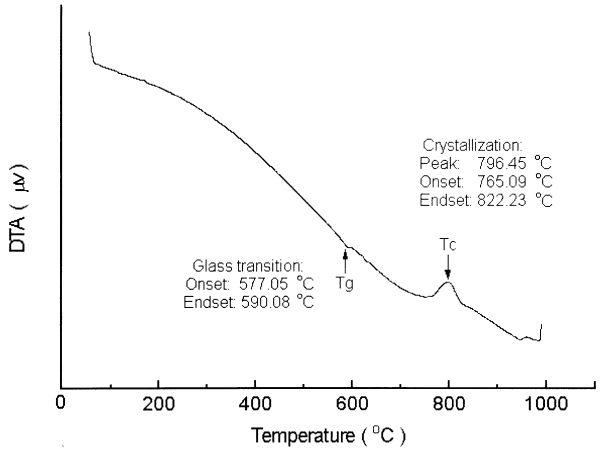

Fig. 1. Differential thermal analysis of calcium phosphate glass.

Table 1
Chemical composition of the glass (mol%)

Oxide	CaO	P_2O_5	TiO_2	MgO	Na_2O
mol%	50.0	40.0	7.0	1.5	1.5

2.2. Sample characterization

Differential thermal analysis (DTA) was carried out in a DTA-50 (Shimadzu) using α-Al_2O_3 powder as a reference. The temperature scanned over a range from room temperature to about 800°C at uniform heating rate of 5°C/min. The values of the transformation temperatures were determined with accuracy of±0.2°C.

X-ray diffraction (XRD) measurements were performed on Siemens D5000 diffractometer using the bulk glass after crystallization. Through flat plate geometry, data were collected from 20 to 50° 2θ, with a step size of 0.05° and a scan speed of 3°/min. Data were collected using a scintillation counter and a graphite-diffracted beam monochromator.

A Jeol scanning microscope (JSM 35C) with energy dispersive X-ray (EDS) spectroscopy (Voyager, Norman Instruments) was used to characterize the surface morphology of the materials after crystallization treatment. Samples were ground and polished down to 1 μm using diamond paste. Polished surfaces were then ultrasonically cleaned with distilled water for 3 min, in order to avoid any preferential dissolution that could interfere with SEM analysis. After soaking in SBF solution, samples were analyzed using SEM and XPS to detect apatite layer formation. An Escalab 200A vacuum generator was used for the quantitative analysis of elements present on the surface, and calibration was performed against an Au $4f_{7/2}$ spectrum at 84.0 eV binding energy. Spectra were referenced to the C_{1S} peak of carbon fixed at 285.0 eV.

2.3. Soaking in SBF solution

Stimulated body fluid (SBF) is an acellular solution with ionic concentration close to that found in human blood plasma,[15,16] as shown in Table 2. A solution of SBF with a pH of 7.25 at 36.5°C, buffered with 50 mM Tris(hydroxymethyl)-aminomethane and 45 mM hydrochloric acid was prepared. Duplicate bulk specimens of the glass-ceramic (10×7×5 mm) were soaked in the solution in sterile polystyrene bottles. A ratio of solution volume to surface area of the specimen was kept at

Table 2
Composition of the simulated physiological solution[16]

Chemicals	Composition (g dm^{-3})
NaCl	7.996
$NaHCO_3$	0.350
KCl	0.224
$K_2HPO_4{\cdot}3H_2O$	0.228
$MgCl_2{\cdot}6H_2O$	0.305
$CaCl_2{\cdot}2H_2O$	0.376
$Na_2SO_4{\cdot}$	0.071
Tris[hydroxymethyl]aminomethane	6.057
HCl, 37.5%	3.68 (cm^3 dm^{-3})

0.1 ml/mm^2. After periods of 1, 2, 4, 6 and 8 weeks of soaking, specimens were removed from SBF solution and carefully washed with distilled and deionized water.

3. Results

Fig. 2 shows XRD results of the glass–ceramic after the two-step heat treatment for nucleation and crystallization. The main crystalline phase precipitated in the microstructure could be clearly assigned to (β-DCP) with CaO/P_2O_5 ratio equal to 2.0 (Ca/P = 1.0), Calcium titanophosphate, $CaTi_4(PO_4)_6$, and/or sodium titanophosphate, $NaTi_2(PO_4)_3$, have also been formed in the structure. These two phases were present in much smaller content than β-$Ca_2P_2O_7$ and have a strong peak overlapping and, therefore, could not be distinguished in the XRD spectrum. It is possible that TiP_2O_7 was also present in very low content and a non-identified phase could also be detected. Kasuga et al. reported a similar occurrence in the structure of glass–ceramics containing 3 wt.% of TiO_2.[7]

These crystalline phases were dispersed in areas of micron size, precipitated in different regions of the glass and could be clearly distinguished at a magnification of ×4000 using SEM, as shown in Fig. 3. EDS analysis also indicated that the light areas of Fig. 3 (denoted by *a*) correspond to β-$Ca_2P_2O_7$ since high CaO content and a Ca/P atomic ratio nearly equal to the theoretical value of 1.0 was identified. The grey areas (denoted by *b*) may correspond to Na-containing phases. It is believed that the dark areas (denoted by *c*) were a result of the partial melting of amorphous phases since they were composed of smaller contents of CaO than the other two areas, and seem to contain most of the MgO and Na_2O added to the $CaO–P_2O_5$ glass. Identical results were obtained by Kasuga et al.[8]

Fig. 4(A) shows the surface of the glass–ceramic after immersion in SBF solution for 4 weeks. Numerous apatite agglomerates were formed on the surface, and the Ca/P molar ratio was determined by EDS analysis as ~1.3, different from the standard Ca/P ratio of 1.67 of stoichiometric hydroxyapatite (HA). After immersion of 8 weeks in SBF solution, apatite crystals still did not cover the whole surface of the specimen. As shown in Fig. 4(B), at a magnification of ×10,000, it was possible to identify the agglomerates composed of very fine needle-like apatite crystallites randomly oriented.

Wide scan X-ray photoelectron spectra of the glass–ceramic before and after the immersion in SBF solution for 8 weeks were recorded for quantitative evaluation of the elements present on the specimen surface, as shown in Fig. 5. O_{1S}, Ca_{2P}, P_{2P}, C_{1S} and Na_{1S} level peaks were detected. A C_{1S} level peak was found on the specimen surface, which should be attributed to unavoidable contamination. A slight decrease in the content of Ca

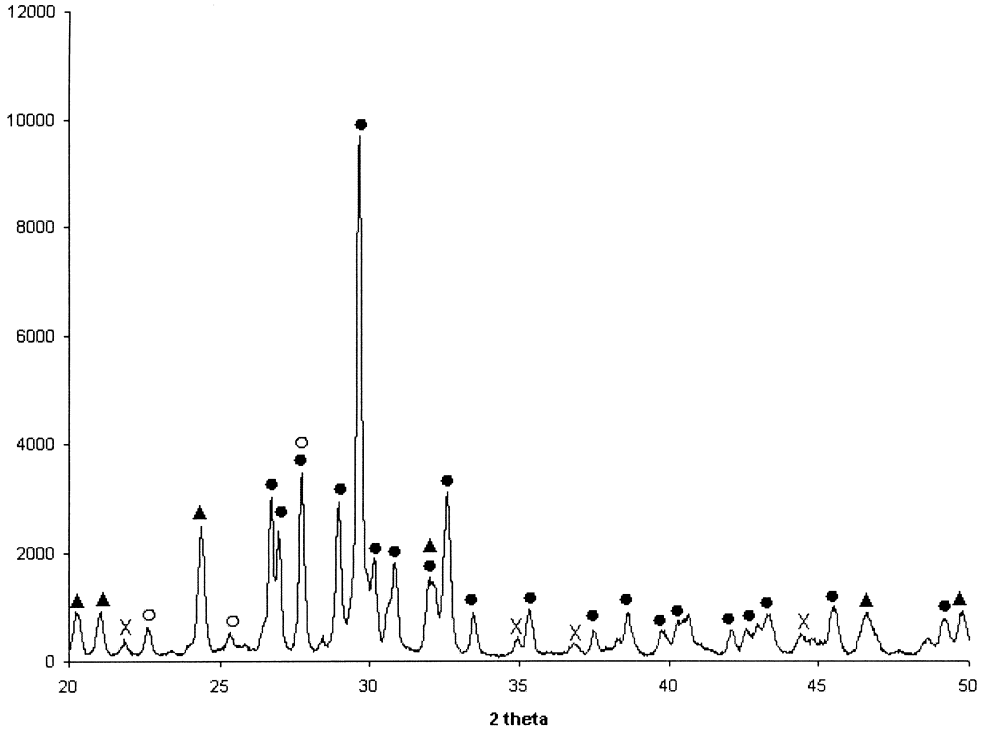

Fig. 2. X-ray diffraction trace (relative intensity vs. 2 theta) of calcium phosphate glass–ceramic after crystallization treatment, (●) β–$Ca_2P_2O_7$, $NaTi_2(PO_4)_3/CaTi_4(PO_4)_6$ (▲), TiP_2O_7 (○) and non-identified phase (x).

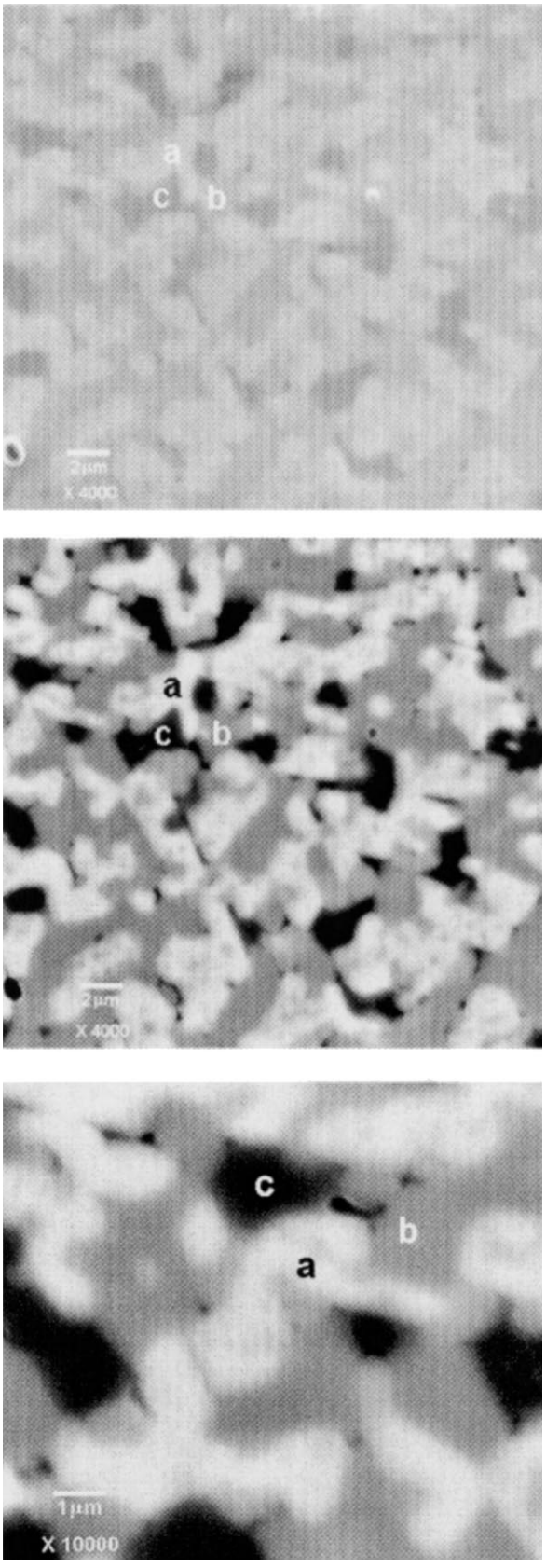

Fig. 3. Scanning electron micrograph of calcium phosphate glass after crystallization. Top — secondary image, ×4000; Middle–back scattering image, ×4000; Bottom — back scattering image, ×10,000.

and P was detected while a much stronger decrease was observed for Na content, which suggests a dissolution of Na-containing phase in simulated physiological media.

Porous microstructure of the glass–ceramic, with interconnective pores of micron size, was achieved through leaching out the soluble phases in diluted hydrochloric acid solution, as shown in Fig. 6.

4. Discussion

Calcium phosphate glasses and glass–ceramics are excellent candidates for bone repair and regeneration biomaterials since calcium and phosphorous are the two main components of bone tissues, although other ions may be present in bone structure, such as Na^+, K^+, Mg^{2+}, F^-, etc. Natural apatite in bone has CaO/P_2O_5 ratio close to 3 although its exact ratio depends upon many factors.[17] However, the difficulty in preparing $CaO\text{-}P_2O_5$ glasses in the pyrophosphate region has been extensively reported.[5] According to phase diagram, calcium phosphate glasses in the binary $CaO–P_2O_5$ system with $CaO/P_2O_5 \geqslant 1.2$ are very difficult to prepare through the conventional melting method, due to the high temperature needed for melting and high tendency to crystallize upon cooling.

Kasuga et al. has been studying the preparation of calcium phosphate glasses and glass–ceramics in the pyrophosphate region for biomedical applications, however, using the powder sintering preparation process.[7] By introducing a small content of TiO_2 (3 mol%) into $CaO–P_2O_5$ glasses followed by crystallization treatment, glass–ceramics with β–$Ca_2P_2O_7$ (β–DCP), β–$Ca_3(PO_4)_2$ (β–TCP) and β–$NaCaPO_4$ phases existing in the microstructure were prepared and they proved to exhibit in vitro bioactivity in simulated body fluid (SBF). In this work, bulk glass–ceramics were prepared using two-step crystallization treatment of $CaO–P_2O_5$ glasses in the pyrophosphate region (CaO/P_2O_5 molar ratio = 1.25), to obtain bioactive crystalline phase such as β–$Ca_2P_2O_7$ (β–DCP) and soluble Na-containing phase. Small contents of Mg^{2+} and Na^+ ions were added to induce and stabilize the crystallization of Na/Ca diphosphates and β–$Ca_3(PO_4)_2$ (β–TCP) phase, as reported by Vogel.[18] However, the β–$Ca_3(PO_4)_2$ (β–TCP) phase could not be detected in the microstructure, probably because of the low content of MgO and TiO_2 added and the low Ca/P ratio of the glass.

Kokubo et al. demonstrated that an apatite layer was formed on the surface of wollastonite-containing glass–ceramic (A-W GC) when it was immersed in an acellular simulated body fluid with ionic concentrations nearly equal to those in human blood plasma within 7 days of immersion.[15] An apatite layer was also reported to occur on hydroxyapatite after 30 days of immersion, while no apatite layer was formed on β–TCP even after

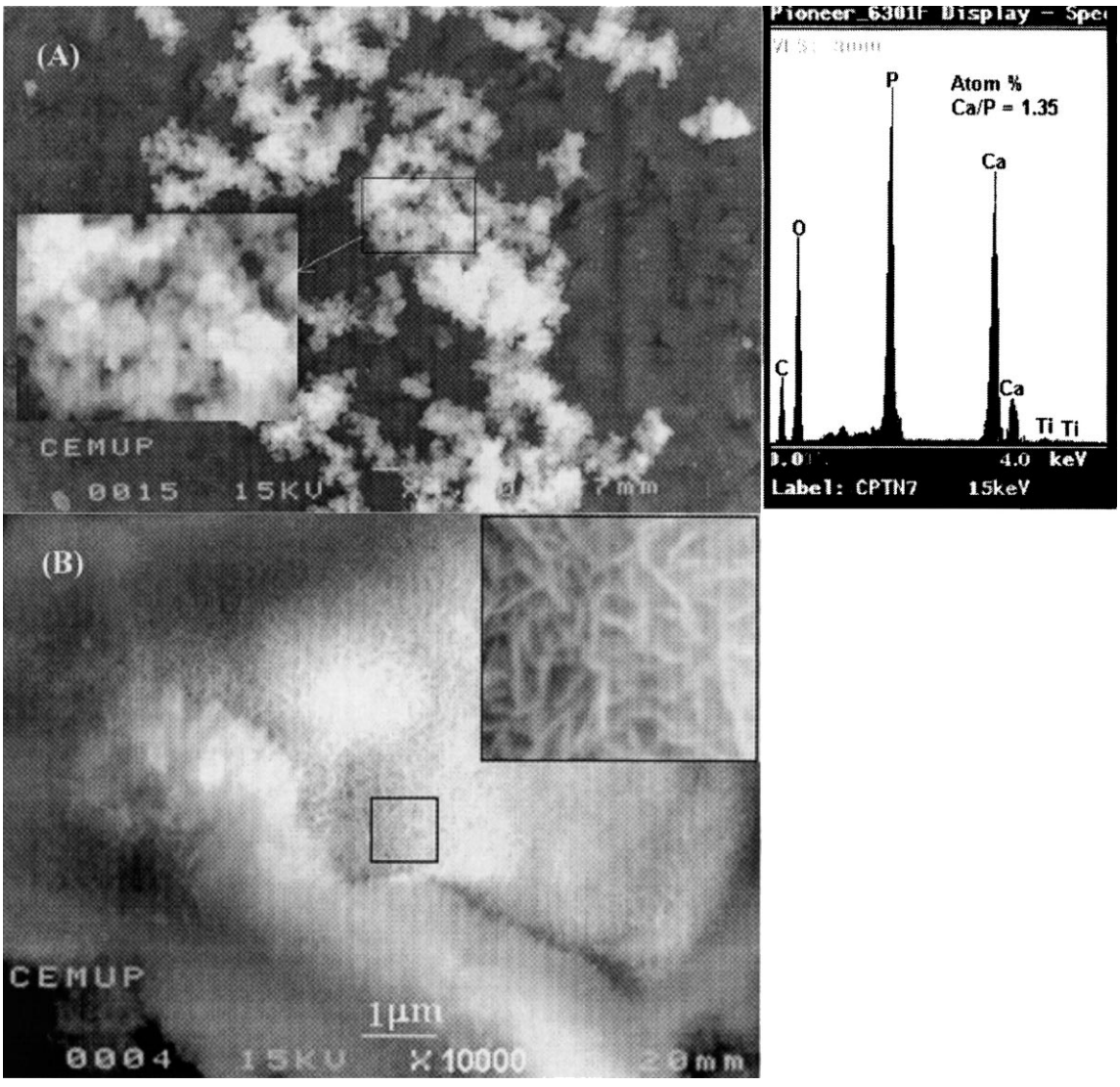

Fig. 4. Scanning electron micrograph and energy dispersive X-ray spectroscopy of calcium phosphate glass-ceramic after immersion in SBF solution for 4 weeks (A) and 8 weeks (B).

120 days.[19] This apatite layer is an in vitro bioactivity indicator of candidate biomaterials. Therefore, in vitro apatite formation on the material's surface is material-dependent and mainly a chemical phenomenon. However, in vivo apatite formation is speculated to be both biological and material-dependent to some extent.[20] Biologically, adsorbed protein or the surrounding bone matrix may promote the apatite precipitation on the surface of biomaterials. There are bioceramics such as β-TCP that seem to bond directly to bone tissues without significant formation of an apatite layer at bone/implant interface.

In the present study, no continuous apatite layer was formed even after 8 weeks of immersion in SBF solution, however, formation of apatite agglomerates containing needle-shaped crystals on the surface of the glass–ceramic suggests that the material is bioactive. The mechanism of apatite crystal formation may be explained by initial dissolution of Na^+, Si^{4+} and Ca^{2+} ions from the surface of the bioactive glass,[21] which increases the degree of supersaturation of the surrounding fluid, resulting in precipitation of new apatite crystals on the surface of the glass–ceramic. However, Mg^{2+} ion existing in glass is known to suppress apatite crystallization in some conditions,[22] and this fact may be one possible reason why no abundant apatite layer was formed on the surface of the glass–ceramic. On the other hand, SBF testing of calcium phosphate with high CaO/P_2O_5 ratio, i.e. resorbable β-TCP, showed that no apatite layer was formed on the surface after 8 weeks of immersion.[19] This glass–ceramic was also designed as drug carrier system. In fact, it seems possible to produce controllable porous structure in physiological media through leaching out the soluble phases, due to the phase separation in the microstructure that was well distinguished at a magnification of ×4000. Although the

pore size obtained is still probably too small for many drug delivery applications, the desirable porous structure may be achieved by control of size and distribution of these phases. The grain boundaries of the soluble crystalline phases dissolved, which led partly to a disintegration of the crystals into free deteriorated grains. Also, the process of dissolution is pH dependent, and strong dissolution (thoroughly porous microstructure, Fig. 6) was observed in 1 N HCl solution. Drug delivery may be realized using these soluble phases as carrier. A time related continuation of the dissolution process may allow continuous release of the drugs to occur. Also, partial pre-dissolution of the glass-ceramic in buffer solution at low pH condition may produce higher pore surface area, which will favor drug adsorption.

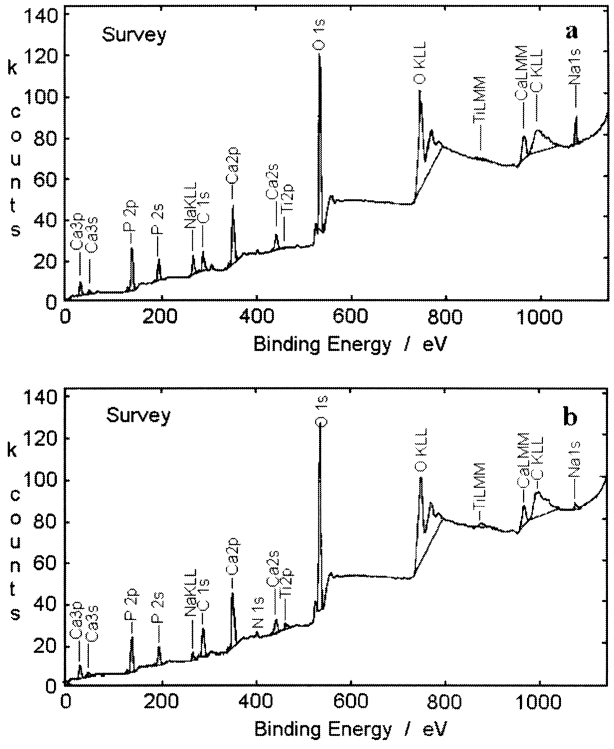

Fig. 5. X-ray photoelectron spectroscopy of calcium phosphate glass-ceramic before (a) and after (b) immersion in SBF solution for 8 weeks.

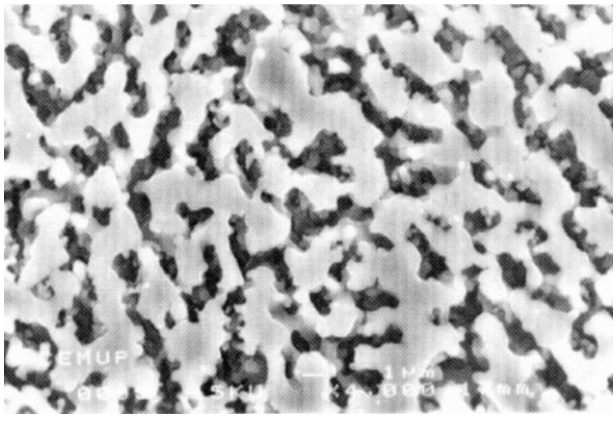

Fig. 6. Scanning electron micrograph of calcium phosphate glass-ceramic after immersion in diluted hydrochloric acid solution (1 N) for 1 min.

5. Conclusions

Bulk calcium phosphate glass and glass–ceramic in the pyrophosphate region (CaO/P_2O_5 molar ratio = 1.25) can be obtained through introducing a small content of additives such as Na_2O, MgO, TiO_2 into the glass. Bioactive and biosoluble phases were precipitated and this glass ceramic seems to have great potential to be used as bone substitute implants and a drug carrier system. No continuous apatite layer was formed after 8 weeks of immersion in SBF solution, although apatite agglomerates were observed on the surface of the glass–ceramic, which is a bioactivity indicator.

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