

# Preparation of calcium pyrophosphate glass-ceramics containing Nb<sub>2</sub>O<sub>5</sub>

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New types of calcium phosphate glass-ceramics were prepared with the addition of Nb<sub>2</sub>O<sub>5</sub> to the pyrophosphate region. The glass-ceramics contained  $\alpha$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>,  $\beta$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, and orthorhombic Nb<sub>2</sub>O<sub>5</sub>. The concentrations of Ca, P and Nb elements released from the glass-ceramics were lower than those released from glass of the same chemical composition. Apatite formed on the surface of the glass-ceramics after soaking in 1.5 simulated body fluid for 7 days. The crystal phases of the glass-ceramics improved the chemical durability and apatite forming ability of the material.

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## 1. Introduction

There has been growing interest in the engineering of soft and hard tissue for use in tissue regeneration. Recent research has focused on the preparation of novel biodegradable bioactive materials. As reported by Hench,<sup>1)</sup> the use of silicate-based bioactive glasses for applications in the field of bone regeneration have gained significant interest over the last three decades. Phosphate-based glass in P<sub>2</sub>O<sub>5</sub>-CaO-Na<sub>2</sub>O systems has higher dissolution properties in aqueous solution than silicate-based glasses. This glass can be synthesized to improve biocompatibility by incorporating other ions.<sup>2),3)</sup> Therefore, phosphate-based glass is expected to have great potential for use with bone regeneration.

Uo et al. reported that the cytotoxicity of P<sub>2</sub>O<sub>5</sub>-CaO-Na<sub>2</sub>O glass was related to glass degradation, the associated pH changes and the ionic concentration of the media.<sup>4)</sup> In the same ternary glass system, glass with lower solubility has been reported to enhance bone cell growth and antigen expression.<sup>5)</sup> This implies that glass with low solubility and a low amount of sodium, which can lead to an increase in the pH when released from the glass, is desirable for bone regeneration applications. Polyphosphate glass often shows more durability than metaphosphate glass because of the reduction of the more readily hydrolyzed Q<sup>2</sup> units in the structure.<sup>6)</sup> We have previously succeeded in preparing calcium pyrophosphate glasses by adding TiO<sub>2</sub> to the pyrophosphate region.<sup>7)</sup> The pH was determined to be neutral after soaking the calcium pyrophosphate glasses in simulated body fluid (SBF), resulting in the formation of an apatite layer on the surface.<sup>8)</sup>

Over the past decade, some types of inorganic ions, such as silicate and calcium ions, have been reported to enhance the bone forming ability of osteoblasts by gene activation.<sup>9),10)</sup> Incorporat-

ing the inorganic ions into calcium pyrophosphate glass is important for enhancing the bone bonding abilities of the materials. Trace amounts of Nb ions have been proposed to promote osteogenic differentiation and mineralization.<sup>11),12)</sup> In cell culture tests, labile surfaces, such as high solubility glass, prevent the formation of physical anchoring by the cells. The addition of metal oxides affects the chemical durability of phosphate glasses and is also expected to affect biocompatibility. It has been proposed that the dissolution rate is significantly controlled by increasing the Nb<sub>2</sub>O<sub>5</sub> content of Nb<sub>2</sub>O<sub>5</sub>-SrO-P<sub>2</sub>O<sub>5</sub> glass.<sup>13)</sup> Niobium was reported to be more effective than titanium for improving chemical durability because of the presence of niobium as a glass network former in a Na<sub>2</sub>O-Al<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub>-Nb<sub>2</sub>O<sub>5</sub>-P<sub>2</sub>O<sub>5</sub> glass system.<sup>14)</sup> We have also reported that the crystallization of the glass after soaking in SBF influences the chemical durability and its apatite-forming ability.<sup>15)</sup>

In this paper, our strategy for improving chemical durability and releasing properties of the inorganic ions from the glass attempted to substitute TiO<sub>2</sub> into the calcium pyrophosphate glass by Nb<sub>2</sub>O<sub>5</sub>. The chemical durability and apatite formation of the samples after soaking in SBF were examined to clarify the importance of the crystallization in the ternary system of CaO-P<sub>2</sub>O<sub>5</sub>-Nb<sub>2</sub>O<sub>5</sub>.

## 2. Experimental procedure

Batch mixtures of 60CaO-30P<sub>2</sub>O<sub>5</sub>-10Nb<sub>2</sub>O<sub>5</sub> glass were prepared using reagent grade CaCO<sub>3</sub>, H<sub>3</sub>PO<sub>4</sub>, and Nb<sub>2</sub>O<sub>5</sub>. The mixtures were melted in Pt crucibles at 1400°C for 30 min in air. The glass was obtained by quenching the melt onto stainless steel. X-ray fluorescence (XRF) analysis revealed the chemical composition of the prepared glass to be 59 mol % of CaO, 28 mol % of P<sub>2</sub>O<sub>5</sub>, and 13 mol % of Nb<sub>2</sub>O<sub>5</sub>, which is almost the same as the batch mixture composition. The differential thermal analysis of the glass showed that the onset of crystallization occurred around 750°C. The prepared glass plates were heated at 800°C for 3 h in air for crystallization.

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The crystal phase of the glass-ceramics was analyzed with an X-ray diffractometer (XRD; PANalytical, X'pert-MPD) using Cu K $\alpha$  radiation, operating at 45 kV, 40 mA. The surface morphology was observed by scanning electron microscopy (SEM; JEOL, JSM-6301F) incorporating X-ray microanalysis using energy dispersive spectrometry (EDS; Noran instruments, Vantage). 5 × 5 × 1 mm glass and glass-ceramic plates were soaked in 20 mL of 1.5 SBF [2.5 mM of Ca<sup>2+</sup>, 213.0 mM of Na<sup>+</sup>, 2.3 mM of Mg<sup>2+</sup>, 7.5 mM of K<sup>+</sup>, 222.5 mM of Cl<sup>-</sup>, 6.3 mM of HCO<sub>3</sub><sup>-</sup>, 1.5 mM of HPO<sub>4</sub><sup>2-</sup>, 0.8 mM of SO<sub>4</sub><sup>2-</sup>, 75.0 mM of (CH<sub>2</sub>OH)<sub>3</sub>CNH<sub>2</sub> and 67.5 mM of HCl with pH 7.4] at 37°C for 7 days to evaluate the apatite-formation on their surfaces. In our preliminary experiment, almost no significant changes in the XRD patterns were observed even after 5 days of soaking in SBF. After soaking in SBF, the plates were gently washed with distilled water and then dried in air atmosphere at room temperature. The morphology of the plates was observed by SEM. The chemical composition of the plates was analyzed by XRF (Seiko Instruments, SEA2200A). 5 mg of powdered glass and glass-ceramics, which were passed through 125–300  $\mu$ m sieves, was soaked in a 15 mL of Tris-buffer solution of 50 mM of (CH<sub>2</sub>OH)<sub>3</sub>CNH<sub>2</sub> and 45 mM of HCl at pH 7.4 at 37°C for 7 days in a polypropylene beaker. The concentration of released ions in SBF and Tris-buffer solution was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Shimadzu, ICPS-7000).

### 3. Results and discussion

**Figure 1** shows the XRD pattern and SEM image of the glass-ceramic plate. The XRD pattern showed peaks corresponding to  $\alpha$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> (PDF card: 09-0345),  $\beta$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> (PDF card: 09-0346), and the Nb<sub>2</sub>O<sub>5</sub> orthorhombic system (PDF card: 27-1003). Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> transforms to an  $\alpha$ -orthorhombic structure at temperatures above 1140°C.<sup>16</sup> When 60CaO–30P<sub>2</sub>O<sub>5</sub>–7Na<sub>2</sub>O–3TiO<sub>2</sub> glass was heated at 800°C in air, the resulting glass-ceramics included  $\beta$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and  $\beta$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>.<sup>17</sup> In this work, the change in the phosphate structure caused by introducing Nb<sup>5+</sup> ions to the glass might be related to the formation of  $\alpha$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>. Dark portions and bright lines were noted from the SEM image and

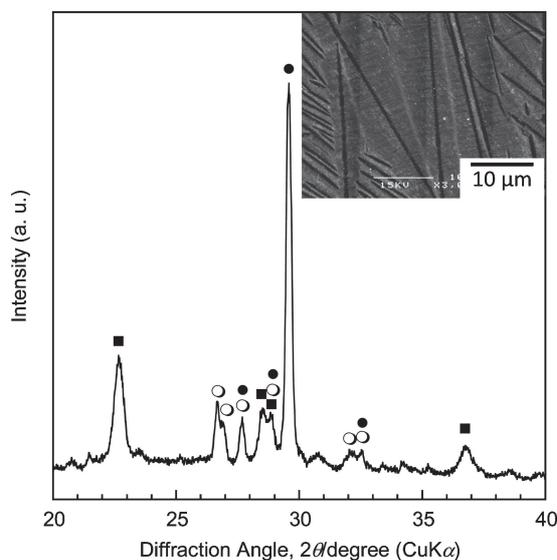


Fig. 1. XRD pattern of the glass-ceramics. (○)  $\alpha$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, (●)  $\beta$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, and (■) orthorhombic Nb<sub>2</sub>O<sub>5</sub>. Inset shows the SEM image of the polished face of the glass-ceramics.

could be caused by the different atomic numbers of the precipitated phase. EDS analysis showed that the bright lines with 42 atom% of Ca, 40 atom% of P, and 18 atom% of Nb, were larger in the Nb content than in the dark portions with 43 atom% of Ca, 43 atom% of P, and 14 atom% of Nb. The Nb<sub>2</sub>O<sub>5</sub> phase identified by XRD is primarily related to the bright lines shown in the SEM image.

**Figure 2** shows the concentrations of released Ca<sup>2+</sup>, P<sup>5+</sup> and Nb<sup>5+</sup> ions after soaking the glass and glass-ceramic powders in Tri-buffer solutions over various periods. These ions were gradually released from the glass. The elemental ratios determined after soaking for 7 days were similar to that of the glass composition. The glass was expected to dissolve uniformly. The concentration of each released ion was lower for the glass-ceramics than the glass. Heat treatment and crystallization of the glass seemed to improve durability. No niobium ion was detected in the Tris-buffer solution after the glass-ceramic was soaked. Niobium in the glass-ceramic was expected to be included in the crystal phase, with orthorhombic Nb<sub>2</sub>O<sub>5</sub> shown in Fig. 1. The concentration of released phosphate ion from the glass-ceramic increased after 1 day and then stabilized. It is proposed that a large amount of phosphate species in the glass is supplied to the formation of the calcium pyrophosphates after the heat treatment. The residual phosphate species in the glassy phase of the glass-ceramic would be released completely within 1 day of the soaking. The number of phosphate ions released from the glass-ceramic was twice that of the calcium ions. Franks et al. reported that the interaction of Ca<sup>2+</sup> ions with the glass network controls glass degradation in the composition range 45P<sub>2</sub>O<sub>5</sub>–*x*CaO–(55–*x*)Na<sub>2</sub>O (*x* = 8–40 mol %).<sup>18</sup> This implies that the crystallization causes a transformation of the phosphate unit structure and interacts with the Ca<sup>2+</sup> ions in the residual glassy phase, leading to changes in the Ca/P releasing ratio.

**Figure 3** shows the XRD patterns of SEM images of the samples after the soaking in 1.5 SBF for 7 days. No peak was observed in the XRD pattern of the glass even after the soaking. On the other hand, newly peak corresponding to hydroxyapatite can be seen in the XRD patterns of the glass-ceramic. The SEM image of the glass plate showed that no deposit formed on the surface even after the soaking. XRF result showed the chemical composition of the glass after 7 days of the soaking to be 59 mol% of CaO, 28 mol% of P<sub>2</sub>O<sub>5</sub>, and 13 mol% of Nb<sub>2</sub>O<sub>5</sub>. However, the glass-ceramic surface shown in Fig. 3(b) was completely covered with leaf-like deposits of hydroxycarbonate apatite, judging from the morphology and XRD pattern. The chemical composition of the glass-ceramic after 7 days of the soaking was determined to be

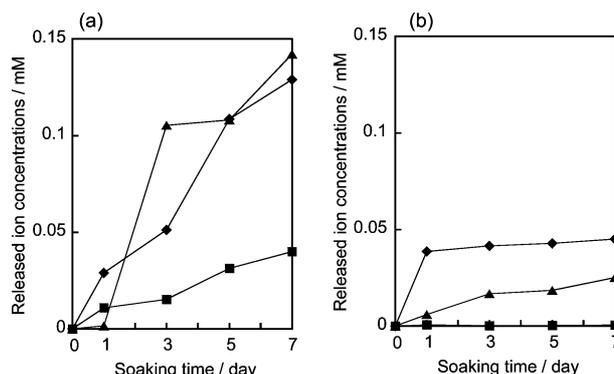


Fig. 2. The releasing concentrations of (▲) Ca<sup>2+</sup>, (◆) P<sup>5+</sup> and (■) Nb<sup>5+</sup> ions from the glasses (a) and glass-ceramics (b) after soaking.

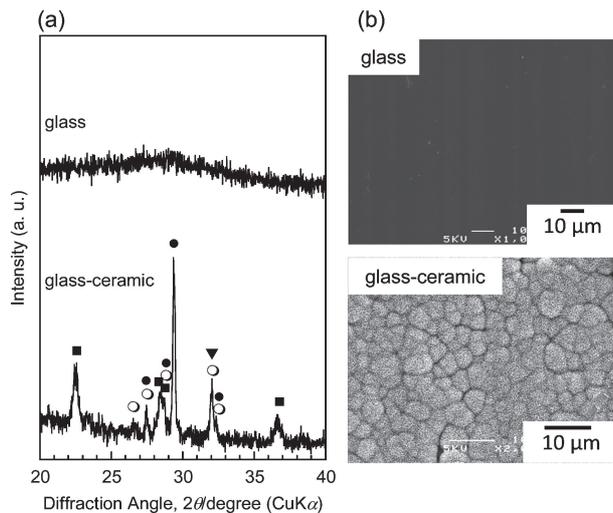


Fig. 3. XRD patterns (a) and SEM images (b) of the surface morphologies for the glass and glass-ceramics after soaking in 1.5 SBF for 7 days. (○)  $\alpha$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, (●)  $\beta$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, (■) orthorhombic Nb<sub>2</sub>O<sub>5</sub>, and (▼) hydroxyapatite.

62CaO–25P<sub>2</sub>O<sub>5</sub>–13Nb<sub>2</sub>O<sub>5</sub> by XRF measurement. The increase in the calcium element originated from the apatite formation on the surface of the glass-ceramic. The concentration of phosphate ion in the SBF after the soaking showed a similar trend on both samples. It increased slightly after 1 day of the soaking and then stabilized. Niobium ions released from two kinds of samples were determined to be less than 2 ppm in the SBF by ICP-AES analysis. The pH in the SBF after the soaking of both samples increased to 7.55 after 7 days. The orthorhombic Nb<sub>2</sub>O<sub>5</sub> has been reported to provide more favorable conditions for apatite nucleation in the SBF than the monoclinic Nb<sub>2</sub>O<sub>5</sub>.<sup>19)</sup> The improved chemical durability and precipitation of orthorhombic Nb<sub>2</sub>O<sub>5</sub> in the glass-ceramics play an important role in the formation of apatite on its surface.

#### 4. Conclusions

Glass-ceramics of the CaO–P<sub>2</sub>O<sub>5</sub>–Nb<sub>2</sub>O<sub>5</sub> system were prepared with the aim to improve chemical durability.  $\alpha$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>,  $\beta$ -Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, and orthorhombic Nb<sub>2</sub>O<sub>5</sub> were precipitated in the glass-ceramics. Nb<sup>5+</sup> ions existed in a crystal phase as orthorhombic Nb<sub>2</sub>O<sub>5</sub> in the glass-ceramics. Precipitation of the crystal phases

in the glass-ceramics improved the chemical durability and their ability to form apatite in SBF.

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