BIOMIMETIC APATITE FORMATION ON CALCIUM PHOSPHATE INVERT GLASSES

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Abstract Bonelike apatite formation on the surface of calcium phosphate glasses was examined in simulated body fluid (SBF) at 37 °C. The apatite particles were deposited on 60CaO·30P2O5·10TiO2 (in mol%) glass containing P2O72- and PO43- ions in 20 days by the soaking, while no apatite particles were deposited on 55CaO·35P2O5·10TiO2 and 50CaO·40P2O5·10TiO2 glasses even by soaking for 30 days. The apatite formation may be influenced by characters of calcium phosphate gel layers, which are formed on the glasses at an initial stage after the soaking in SBF.

INTRODUCTION

A limited kind of ceramics and glasses implanted into bone defects bonds directly to living bone without encapsulation by a fibrous tissue (i.e., bioactivity). They form a carbonate-containing hydroxyapatite layer on their surfaces in the living body and bond to living bone through the apatite layer. The layer is very similar to the apatite in the bone in its composition and structure (bonelike apatite). The apatite can be formed biomimetically on bioactive materials even in simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma. For the biomimetic apatite formation on materials, chemical species for induction of the nucleation should be included at the surfaces. And also, increase in the supersaturation to the apatite in body fluid or SBF has an effect on the formation.

Bonelike apatite layer is formed also on bioactive silicate glasses and glass-ceramics such as Bioglass® and Cerabone A-W® after soaking in SBF at 37 °C. It was proposed that a hydrated silica plays a role in forming the apatite layer on the
surfaces of bioactive silicate glasses, glass-ceramics or gels. There were, however, almost no reports on the apatite formation on phosphate glasses without silica, to our knowledge.

Recently, our research group reported that calcium phosphate glasses in the pyrophosphate region can be prepared successfully by addition of a small amount of TiO$_2$\textsuperscript{7} and found for the first time that bonelike apatite can be formed on 60CaO·30P$_2$O$_5$·10TiO$_2$ (in mol%).\textsuperscript{8} In the present work we examined the influence of CaO/P$_2$O$_5$ ratio in the glasses on the ability to form bonelike apatite on the surface.

**EXPERIMENTAL PROCEDURE**

Phosphate glasses were prepared using compositions of $x$CaO·(90 − $x$)P$_2$O$_5$·10TiO$_2$ ($x = 45$ − 60) in mol%. The mixture of starting materials such as reagent grade CaCO$_3$, H$_3$PO$_4$ (85% liquid), and TiO$_2$ was stirred to make a slurry. After drying the slurry, it was melted in a platinum crucible at 1350 °C for 0.5 h. The melt was poured onto a stainless-steel plate and quickly pressed using an iron plate, resulting in formation of glasses with thickness of 0.5 ~ 1 mm. The structure of the glasses was examined by magic angle spinning $^{31}$P nuclear magnetic resonance (MAS-NMR) spectroscopy. Chemical shifts in the spectra were measured relative to 85% H$_3$PO$_4$.

xCaO·(90 − $x$)P$_2$O$_5$·10TiO$_2$ glasses with a surface area of 50 ~ 200 mm$^2$ were soaked in 100 mL of SBF (Ca$^{2+}$; 2.5, Mg$^{2+}$; 1.5, Na$^+$; 142.0, K$^+$; 5.0, Cl$^-$; 148.8, HCO$_3^-$; 4.2, and HPO$_4^{2-}$ including (CH$_2$OH)$_3$CNH$_2$; 50 and HCl; 45.0, in mM) at pH 7.40 at 37 °C. After the soaking, the surface was examined by x-ray diffraction (XRD) analysis and Fourier transform infrared reflection spectroscopy (FT-IRRS), and also it was observed by using a scanning electron microscope (SEM) incorporating an energy dispersive spectrometer (EDS).

The glasses, which were pulverized into granules with a diameter of 45 ~ 150 μm, were used in order to evaluate their chemical durability. The granular sample (0.2 g) was soaked in SBF of 20 mL at 37 °C. After the soaking, SBF was filtrated to remove the glass particles and new depositions. Amounts of Ca$^{2+}$, P$^{5+}$, and Ti$^{4+}$ ions in the SBF were determined by an inductively coupled plasma atomic emission spectroscopic (ICP-AES) analysis.
RESULTS AND DISCUSSION

Figure 1 shows $^{31}$P MAS-NMR spectra of the phosphate glasses. Bonding of PO$_4$ tetrahedra is described as $Q^n$ units where "n" is the number of bridging oxygens to neighboring tetrahedra. In the glasses of $x \leq 50$, peaks for $Q^2$ and $Q^1$ units are observed. The glasses consist predominantly of the metaphosphate group. In the glass of $x = 55$, only the peak for the $Q^1$ unit is seen. When the CaO content is 60 mol%, an additional signal appears at 2.5 ppm for the $Q^0$ unit. These signals demonstrate the existence of the pyrophosphate and orthophosphate groups without the metaphosphate group. The calcium phosphate glasses containing CaO of $\geq 55$ mol% with TiO$_2$ of 10 mol% can be called "invert glasses" containing P$_2$O$_7^{2-}$ and PO$_4^{3-}$ ions. It is not easy to prepare the glasses of $x > 60$ using the present process.

XRD patterns and SEM photos of the surface of the glasses of $x = 50, 55$ and 60 after the soaking for 20 days are shown in Figure 2. We can see a significant change in the surface of the glass of $x = 60$. XRD peaks of apatite appeared and numerous depositions of leaf-like particles were observed via SEM. The depositions were confirmed, using FT-IRRS, to contain carbonate ions. The depositions were found to be a calcium phosphate phase with Ca/P $\approx 1.5$ in atomic ratio, including a small amount of magnesium, using EDS analysis. The Ca/P ratio is much smaller than the stoichiometric ratio for apatite (1.67).
than that of stoichiometric hydroxyapatite and titanium is not included in the layer; the layer consists of bonelike apatite phase. In XRD patterns of the glasses of $x = 55$ and 50, however, no apatite peaks except an unknown peak are seen. No apatite formation is observed via SEM. Even after the soaking for 30 days, new calcium orthophosphate crystals were not formed on the glasses of $x = 55$ and 50. We found that only $60\text{CaO} \cdot 30\text{P}_2\text{O}_5 \cdot 10\text{TiO}_2$ glass with the orthophosphate group has the apatite formation ability on the surface in SBF. Additional SEM observation showed that the new apatite starts to form on the surface of the glass after soaking for 10 days.

FIGURE 2. (a) XRD patterns and (b-d) SEM photos after soaking the $x\text{CaO} \cdot (90-x)\text{P}_2\text{O}_5 \cdot 10\text{TiO}_2$ ($x = 50, 55$ and 60) glasses for 20 days at 37 °C in SBF.
Figure 3 shows concentrations of Ca$^{2+}$ and P$^{5+}$ ions in SBF after soaking the granules of $60$CaO·$30$P$_2$O$_5$·10TiO$_2$ and $50$CaO·$40$P$_2$O$_5$·10TiO$_2$ glasses. Their amounts after the soaking of the glasses were found to be over an order of magnitude smaller than those from the metaphosphate glass. The amount of Ti$^{4+}$ ions in SBF was below analytical limit of $<10^{-3}$ mM. As dissolution of phosphorus ions from the glasses is suppressed extremely, no local lowering of the pH of the solution around the glasses would occur.

Our earlier work reported that intermediate zone (0.5 ~ 2 μm thick) including almost no titanium exists between the $60$CaO·$30$P$_2$O$_5$·10TiO$_2$ glass and the newly deposited apatite phase. The zone was suggested to be a new gel layer formed prior to the apatite deposition in SBF. The amounts of calcium and phosphorus ions in SBF dissolved from the $60$CaO·$30$P$_2$O$_5$·10TiO$_2$ and $50$CaO·$40$P$_2$O$_5$·10TiO$_2$ glasses did not change almost or decreased very slightly. The calcium and phosphorus ions dissolved may be adsorbed on their surface and subsequently form a thin gel layer on their surface. Tanahashi, et al. showed that a PO$_4$H$_2$ group is an inducer for the apatite formation in SBF. It seems that the calcium phosphate gel layer formed on the $60$CaO·$30$P$_2$O$_5$·10TiO$_2$ glass surface plays an important role in the apatite formation in SBF; the layer may provide the nucleation sites of the apatite.

$60$CaO·$30$P$_2$O$_5$·10TiO$_2$ glass having the orthophosphate and pyrophosphate groups deposits the new apatite particles on its surface in SBF, while $50$CaO·$40$P$_2$O$_5$·10TiO$_2$ glass having the metaphosphate structure do not deposite the apatite. The amount of calcium ions dissolved from $60$CaO·$30$P$_2$O$_5$·10TiO$_2$ glass is slightly larger.
than that from $50\text{CaO} \cdot 40\text{P}_2\text{O}_5 \cdot 10\text{TiO}_2$ glass. The character of the gel layer formed on $60\text{CaO} \cdot 30\text{P}_2\text{O}_5 \cdot 10\text{TiO}_2$ glass may be different from that on $50\text{CaO} \cdot 40\text{P}_2\text{O}_5 \cdot 10\text{TiO}_2$ glass. Biomimetic apatite formation on the phosphate glasses is supposed to be influenced by various factors such as basicity and amount of the functional groups attributed to the nucleation at their surface.

In summary, $60\text{CaO} \cdot 30\text{P}_2\text{O}_5 \cdot 10\text{TiO}_2$ glass containing the orthophosphate and pyrophosphate groups was found to form a bonelike apatite layer on its surface in SBF. Bioactivity can be controlled by optimizing the design of a glass composition even using phosphate glasses without silica. Such glasses may be promising materials for application to bioactive coatings on metals, bioactive cements, or bone fillers.

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References