APATITE FORMATION ON CaO,SiO₂-BASED GLASS-CERAMICS IN A SIMULATED BODY FLUID

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Keywords: Apatite, Bioactivity, Glass-ceramics, Simulated body fluid.

Abstract: The formation of a bone-like apatite layer is essential for glasses and glass-ceramics to show osteoconductivity, i.e., a bone-bonding property, when they are implanted in bony defects. Glasses in the CaO–SiO₂ binary system show promise in being able to deposit apatite after exposure to a body fluid, since these glasses can release Ca²⁺ ions to increase the degree of supersaturation of the surrounding body fluid with respect to apatite, as well as having the ability to form silanol groups that can induce heterogeneous nucleation of apatite on their surfaces. In this paper, we review different types of glass-ceramics that are mainly composed of CaO and SiO₂ for their potential to form apatite in a simulated body fluid that has similar ion concentrations to those found in human blood plasma.

(Received April 17, 2006; Accepted December 15, 2006)

INTRODUCTION

Bones play an important role in supporting the human body, as well as protecting the internal organs, and they help preserve the body’s calcium and phosphate ions. Damage to bone by disease or by accident results in a significant decrease in the quality of an individual’s life, because bones are the key to an individual’s mobility. Bone defects have been repaired using treatments involving substitution with autogenous and allogenic bones. Autogenous bone is graft-transferred from another part of the skeleton of the same patient, and treatment with autogenous bones causes damage to the otherwise healthy bones of the patient. In addition, there is a limitation to the available bones in a patient for autogenous grafting. Allogenic bone is graft-transferred from another subject, and the use of allogenic bones sometimes leads to infection. In addition, the availability of allogenic bone is also limited. Therefore, the use of artificial material is an important topic of study for repairing bone defects, and an artificial material for substituting for natural bone is denoted as “artificial bone”.

Some ceramics have been already used as artificial bone materials in clinical applications to repair bone defects. Ceramic biomaterials for bone repairing are usually categorized into three types, according to the reaction in the body: bioinert ceramics, bioactive ceramics, and bioresorbable ceramics, as shown in Table 1. Bioinert ceramics are used as femoral balls in total hip joint replacements because of their high chemical durability and their high wear resistance. Bioresorbable ceramics are used as bone substitutes and as potential scaffolds for bone regeneration.

Bioactive ceramics are used as bone substitutes because they have an attractive bone-bonding ability. To isolate artificial materials from the surrounding bone, they are usually encapsulated by a noncalcified fibrous tissue when implanted into a bone defect. In contrast, bioactive ceramics can be in direct contact with living bone, and can form strong bonds. This bone-bonding property is an outstanding advantage when bioactive ceramics are used as bone substitute materials.

Bioactive ceramics were discovered in the early 1970s by Hench et al.²,³ They showed that some glasses in the Na₂O-CaO-SiO₂-P₂O₅ system spontaneously bond to living bone without forming a fibrous tissue around them, and the glasses that show bone-bonding properties were named Bioglass⁶. Since the discovery of Bioglass⁶, various types of ceramic, such as sintered hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂)³,⁵ and glass-ceramic A-W containing crystalline apatite and wollastonite, have been shown to have bone-bonding properties. The ability to form bone tissue on a bioactive ceramic is known as osteoconductivity, and sometimes simply as “bioactivity”. Some osteoconductive ceramic materials have been subjected to clinical applications as bone substitutes in orthopedic and dental medicine.

Porous bodies made of bioactive ceramics have also been used clinically as bone fillers, since cells and tissue can enter the pores in these materials. They have attracted much attention as scaffold materials for bone regeneration in tissue engineering, where they have incorporated drugs or osteoinductive factors.⁸ A skeleton of porous bioactive glasses and glass-ceramics is useful, since the bioactivity and thermal properties of these materials can easily be controlled by varying the composition of the base
This paper reviews the mechanism of osteoconduction of bioactive glasses and the design of some types of glass-ceramics based on this mechanism.

**BONE-BONDING MECHANISM OF BIOACTIVE GLASSES**

The bioactive glasses form a bone-like apatite layer on their surfaces and bond to living bone through this apatite layer when they are implanted into bony defects. Therefore, it is believed that the essential requirement for artificial materials to exhibit bioactivity is the formation of an apatite layer on their surfaces in a body environment.\(^9,^{10}\) A schematic representation of the bonding mechanism of bioactive ceramics is shown in Fig. 1. Kokubo et al. reported that the apatite formation can be reproduced in a simulated body fluid (SBF)\(^11,^{12}\) whose inorganic ion concentrations are similar to those of human blood plasma. The ion concentrations of SBF are shown in Fig. 2, and the pH of SBF is adjusted to be around pH = 7 at 36.5 °C using a tris buffer (50 mol·m\(^{-3}\) of tris(hydroxymethyl)aminomethane and approximately 45 mol·m\(^{-3}\) of hydrochloric acid). The SBF does not contain any cells or proteins, and this indicates that the formation of the apatite layer is the result of a chemical reaction between the ceramic and the body fluid, where the chemical reaction governs the bioactivity of the ceramic.

Ohtsuki et al.\(^13\) reported on suitable compositions of bioactive glasses by evaluating apatite formation on the surface of glasses in the CaO–SiO\(_2\)–P\(_2\)O\(_5\) system after exposure to SBF. The results are summarized in Fig. 3. This data shows that an apatite layer is formed not in the region mainly composed of CaO and P\(_2\)O\(_5\), but in the region mainly composed of CaO and SiO\(_2\). Although both human body fluid and SBF contain calcium and phosphate ions that are supersaturated with respect to apatite, spontaneous precipitation of apatite does not occur in a human body fluid or SBF under normal conditions, because the energy barrier to induce apatite nucleation is too high for spontaneous precipitation under normal conditions. The reason why apatite is formed preferentially on a glass surface that is mainly composed of CaO and SiO\(_2\) is that the Ca\(^{2+}\) ions released from the glass increases the degree of supersaturation with respect to apatite of the surrounding fluid, and the Si–OH groups of the hydrated silica gel formed on the surface induce heterogeneous nucleation of apatite.\(^14,^{15}\) Figure 4 shows a schematic representation of the mechanism of apatite formation on CaO–SiO\(_2\) glasses. Once apatite nuclei form, crystals grow spontaneously by consuming calcium and phosphate ions from the surrounding body fluid that is already supersaturated with respect to apatite to form an apatite layer.

Therefore, CaO, SiO\(_2\)-based glasses are expected to be useful bone-repairing materials because they are expected to show high bioactivity. A glass-ceramic system has advantages compared with a solely glass system because the precipitated crystalline phase may provide preferred properties to the materials, such as a high mechanical strength.
Fig. 2. Evaluation of bioactivity using SBF.

Fig. 3. Compositional dependence of apatite formation in CaO–SiO$_2$–P$_2$O$_5$ glasses in SBF.$^{13}$
Soaking period = 30 d. Key: ⊙ = apatite formation, ⊙ = no apatite formation, and ⊙ = severe dissolution.

Fig. 4. Mechanism of apatite formation on CaO–SiO$_2$ glass in a body environment.$^{14}$
MgO–CaO–SiO\textsubscript{2} GLASS-CERAMICS CONTAINING WOLLASTONITE AND DIOPSIDE

Glasses in the CaO–SiO\textsubscript{2} binary system act as basic components when producing bioactivity. However, the reactivity of 50CaO·50SiO\textsubscript{2} mol% glass in a body environment is so high that a thick silica gel layer is observed between the apatite layer and the glass after soaking in SBF. This thick silica gel layer is not desirable, since its mechanical strength is low, and this may affect the bonding between the glass and bone. Therefore, a design in which the glass does not form a thick silica gel layer is required.

Magnesium is a major inorganic element that exists in a body fluid, and has already been used in bioactive glass-ceramics, such as glass-ceramic A-W. When CaO is partially replaced with MgO, a decrease in the thickness of the silica gel layer between the apatite layer and the glass is observed.\textsuperscript{16} The glass with a composition of 10MgO·40CaO·50SiO\textsubscript{2} mol% forms apatite in SBF within a period of 3 d, and the apatite layer formed is in direct contact with the glass substrate without the formation of a thick silica gel layer between the apatite and the glass. This means that a strong bonding of the apatite and the glass substrate is expected. Therefore, it is expected that the 10MgO·40CaO·50SiO\textsubscript{2} mol% composition is useful for designing glass-ceramics. Based on this idea, Ohtsuki \textit{et al.}\textsuperscript{17} synthesized a glass with the nominal composition of 10MgO·40CaO·50SiO\textsubscript{2} mol% using a conventional melt–quenching technique. A slurry consisting of the pulverized glass and ultrapure water was loaded in a polyurethane sponge, which was then heated to various temperatures to obtain porous glass-ceramics. A porous body with both a high bone-bonding ability and biodesorbability is expected to be a useful scaffold for use in bone regeneration. Figures 5 and 6 show the powder X-ray diffraction (XRD) patterns and scanning electron microscope (SEM) micrographs of the resultant porous glass-ceramics. Porous bodies with continuous pores of about 500 \(\mu\)m in diameter were obtained after sintering and crystallization. Chang \textit{et al.}\textsuperscript{18} reported that pores 300–500 \(\mu\)m in diameter in hydroxyapatite samples were effective for bone ingrowth. Therefore, this porous structure is expected to be useful for the invasion of cells and new bone when these glass-ceramics are implanted into bone defects. Precipitation of parawollastonite and diopside was observed after heat treatment at temperatures above 900 °C. The amount of precipitated diopside increased with increasing heat treatment temperature. The synthesized glass-ceramics formed apatite on their surfaces after soaking in SBF, as shown in Fig. 7. Consequently, glasses in the MgO–CaO–SiO\textsubscript{2} system allow for easy production of porous glass-ceramics with bioactivity and biodegradation using a conventional sintering process.

![Fig. 5. Powder XRD patterns of porous glass-ceramics heat-treated at various temperatures.\textsuperscript{17}](image1)

![Fig. 6. SEM micrographs of the inside of porous glass-ceramics heat-treated at various temperatures.\textsuperscript{17}](image2)
MgO–CaO–SiO₂–P₂O₅ GLASS-CERAMICS CONTAINING WHITLOCKITE AND DIOPSIDE

Tricalcium phosphate (3CaO-P₂O₅, TCP) is known as a bioresorbable ceramic, and is already used clinically as an important bone-repair material. However, it is not easy to control its bone-bonding ability and bioresorbability, because TCP is a crystalline ceramic. The combination of glass-based ceramics with TCP may lead to novel bioactive and bioresorbable materials for use as bone substitutes. In order to control the bioactivity and bioresorbability of ceramics, glasses and glass-ceramics are useful because their chemical and thermal properties can easily be controlled by varying their composition. As discussed, glasses in the CaO–SiO₂ binary system act as basic components when producing bioactivity. It has been reported that diopside (CaO-MgO-2SiO₂) ceramics show the potential for making direct contact with bone, and they have a high mechanical strength. Glass-ceramics containing TCP and diopside would therefore be candidate materials for showing high bioactivity and high mechanical strength in the initial stages after implantation, followed by an appropriate degradation during bone regeneration. Therefore, it is worth investigating the feasibility of bioactive glass-ceramics derived from glasses in the 3CaO-P₂O₅–CaO-MgO-2SiO₂ system, and so Kamitakahara et al. prepared glass-ceramics containing TCP and diopside from 3CaO-P₂O₅–CaO-MgO-2SiO₂ glasses.

Glass-ceramics with the compositions of x(3CaO-P₂O₅)·(100–x)(CaO-MgO-2SiO₂) were prepared, where x = 0, 38, 50, or 60 mass%, as shown in Fig. 8 (Samples 100D, 38T62D 50T50D, and 60T40D, respectively). Pulverized glasses of each composition were compacted and heated to obtain the bulk glass-ceramics. Figure 9 shows the powder XRD patterns of the dense glass-ceramics sintered at temperatures of 1,000 and 1,200 °C.

When the glass-ceramics were sintered at 1,000 °C, whitlockite (β-TCP), oxyapatite, and diopside were precipitated in glass-ceramic Samples 60T40D and 50T50D, oxyapatite and diopside in Sample 38T62D, and only diopside was precipitated in Sample 100D. The diopside content increased with the increasing diopside component in the glass composition. When the glass-ceramics were sintered at 1,200 °C, both β-TCP and diopside were precipitated in Samples 60T40D, 50T50D, and 38T62D, while only diopside was precipitated in glass-ceramic Sample 100D. The diopside content decreased with increasing TCP content in the glass composition, while the β-TCP content increased with increasing TCP content in the glass composition. Sintering at 1,200 °C led to the formation of β-TCP and diopside in glass-ceramic Samples 60T40D, 50T50D, and 38T62D. Oxyapatite was precipitated at a lower temperature than diopside and β-TCP in Samples 60T40D, 50T50D, and 38T62D. The oxyapatite gradually transformed to β-TCP with increasing diopside precipitation, as shown in Equation (1), because the CaO content of the residual glass phase decreased with increasing diopside precipitation.

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Ca_{10}(PO_4)_6O \rightarrow 3(Ca_3(PO_4)_2) + CaO \quad (1)
\]
Fig. 8. Phase diagram of the 3CaO-P_2O_5–CaO-MgO-2SiO_2 system,\textsuperscript{22} and the composition of the prepared glasses.

Fig. 9. Powder XRD patterns of the dense glass-ceramics sintered at 1,000 and 1,200 °C.\textsuperscript{21}

After the glass-ceramics sintered at 1,200 °C were soaked in SBF, fine precipitates were observed on the surface of glass-ceramic Samples 60T40D and 50T50D within a period of 3 d, and on Sample 38T62D within a period of 7 d, as shown in Fig. 10. These precipitates were assigned to bone-like apatite from their XRD patterns. The formation of apatite on Sample 100D was observed after a period of 14 d after soaking in SBF. The reason why the apatite-forming ability increased with increasing TCP content in the glass composition is attributed to the ease of release of calcium ions from the residual glassy phase in the glass-ceramics into SBF, as the dense sintered β-TCP shows a low ability for inducing apatite formation in the normal SBF.\textsuperscript{23} However, there remains the issue of the dissolution rate of the glass-ceramics in relation to the degree of sintering. In the case of glass-ceramic Samples 60T40D and 50T50D, pores were observed to be present, even in the dense glass-ceramics, and it is suggested that these have a large surface area, and may provide for the easy release of calcium ions. Therefore, further examination is required to clarify the effect of the dissolution rate of the glassy phase.

Porous glass-ceramics with continuous pores of about 500 µm in size were successfully prepared with the above compositions when a slurry composed of the glass powders and ultrapure water was loaded in a polyurethane sponge and heated (Fig. 11). These porous glass-ceramics containing β-TCP and diopside are expected to be useful as scaffold materials for bone repair.
As discussed above, the bioactivity of ceramics is governed by the formation of an apatite layer from a reaction between the ceramic and a body fluid. Moreover, the bioresorption of the ceramics is also governed by a chemical reaction. Therefore, it is important to control this chemical reaction in the design of bioactive glass-ceramics. It is expected that the addition of zinc oxide to a bioactive glass-ceramic may control the reaction between the glass-ceramic and surrounding body fluid. Zinc oxide was selected, as zinc is an essential trace element that shows a stimulatory effect on bone formation. The zinc ions released from a glass-ceramic may enhance bone regeneration in a bony defect. In a study on ceramics designed to release zinc ions, Ito et al. recently developed calcium phosphate ceramics containing zinc. However, they used polycrystalline ceramics, and so the range where zinc could be incorporated into their samples was limited, and the behavior of their materials is expected to be different from that of glass-based materials. Among the bioactive ceramics, glass-ceramic A-W shows a high bioactivity and high mechanical strength. The effect of the addition of ZnO to CaO–SiO₂–P₂O₅–CaF₂ glass-ceramics, whose compositions were based on a modification of CaO–ZnO–SiO₂–P₂O₅–CaF₂ GLASS-CERAMICS CONTAINING APATITE AND WOLLASTONITE
The composition of the glass studied was based on a modification of glass-ceramic A-W. Glasses with the composition $x\text{ZnO}\cdot(57.0-x)\text{CaO}\cdot35.4\text{SiO}_2\cdot7.2\text{P}_2\text{O}_5\cdot0.4\text{CaF}_2$ were prepared (where $x = 0.0, 0.7, 3.6, 7.1,$ or $14.2$ mol%) using a conventional melt–quenching technique (Samples Zn0, Zn0.7, Zn3.6, Zn7.1, and Zn14.2, respectively). Compacts of the glass powders were heated to a temperature of 930 °C for 4 h for sintering and crystallization. Peaks ascribed to apatite and parawollastonite were observed in all the glass-ceramic samples, as shown in Fig. 12. In Sample Zn14.2, a peak ascribed to cyclowollastonite was also observed. These results indicate that glass-ceramics containing apatite and wollastonite were obtained using this procedure.

Figure 13 shows SEM micrographs of the surfaces of the glass-ceramics after soaking in SBF for a period of 7 d. Precipitates composed of fine particles were observed on glass-ceramic Samples Zn0 and Zn0.7, but they were not observed on Samples Zn3.6, Zn7.1, and Zn14.2. These precipitates exhibited broad apatite peaks in their thin-film XRD patterns. Apatite was newly formed on glass-ceramic Samples Zn0 and Zn0.7 in SBF, but it was not formed on Samples Zn3.6, Zn7.1, and Zn14.2.

Changes in the element concentrations of SBF after soaking the glass-ceramics are shown in Fig. 14. The calcium concentration increased in the initial stages for all the glass-ceramic samples. This increase in calcium concentration was induced by the release of calcium ions from the glass-ceramic samples. The decrease in calcium concentration in the later stages for Samples Zn0 and Zn0.7 is because of the consumption of calcium ions through apatite formation on their surfaces. The phosphorus concentration decreased when glass-ceramic Samples Zn0 and Zn0.7 were soaked in SBF, whereas it remained almost constant when glass-ceramic Samples Zn3.6, Zn7.1, and Zn14.2 were soaked in SBF. This decrease in phosphorus concentration was because of the consumption of phosphate ions through the formation of apatite on the surfaces of glass-ceramic Samples Zn0 and Zn0.7.

The increase in silicon concentration indicates the release of silicate ions from the glass-ceramic samples. The release of silicon from the glass-ceramics decreased significantly with increasing ZnO content. This indicates that the reaction between the glass-ceramics and SBF was suppressed, and the formation of silanol groups was also suppressed by the addition of ZnO. It is speculated that the chemical durability of the glass-ceramic samples was improved by the addition of ZnO, since ZnO is an amphoteric oxide, and has a very low solubility in SBF. The suppression of the formation of silanol groups would lead to the suppression of apatite formation on the glass-ceramic samples. Ohtsuki et al. previously reported that the addition of even a small amount of Al$_2$O$_3$ to a bioactive CaO–SiO$_2$ glass also suppresses the release of calcium ions from the glass, and decreases its bioactivity. However, when the content ZnO was low, the glass-ceramic samples still showed the apatite-forming ability. The reaction between a glass-ceramic sample and a body fluid governs its bioactivity, and also its biodegradability. This suggests that the biodegradability of a glass-ceramic can be controlled to some extent without loss of its bioactivity.

The release of zinc was detected in glass-ceramic samples containing 3.6 mol% or more of ZnO, and the amount of zinc increased with increasing ZnO content. The amount of zinc released was much less than that of silicon released. In a previous study, Sogo et al. reported that a calcium phosphate ceramic containing zinc, which released about 0.01 mol·m$^{-3}$ of zinc in a buffer solution at pH = 4.9 over a period of 7 d, effectively enhanced bone formation. The zinc concentration obtained in the study was a little lower than that reported by Sogo et al., since this study examined the release of zinc at pH = 7.25, but the observed values are consistent with those reported by Sogo et al. This suggests that these glass-ceramics may also enhance bone formation by the release of zinc when they contain an appropriate amount of ZnO. From the above results, a zinc content between Samples Zn0.7 and Zn3.6 may provide a composition that has the apatite-forming ability and appropriate zinc release rate.

![Fig. 12. Thin-film XRD patterns of the surfaces of the glass-ceramics.](image-url)
SUMMARY

Glasses in the CaO–SiO₂ binary system have a high potential for bioactivity. By modifying this system, we can obtain various bioactive glass-ceramics with controlled properties, such as bioresorbability and ion release. Glass-ceramics that are mainly composed of CaO and SiO₂ are expected to be useful as bone-repairing materials.

REFERENCES