

Apatite-forming ability and antibacterial property of Ag-CaO-SiO₂/polyetherether ketone composites

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Ceramic biomaterials have been used to recover the lost functions of the damaged bones by direct fusion with the bones. CaO-SiO₂ system has been known as a bioactive material that has an ability to form apatite on the surface of the bones inside the body fluid. Applications of biomaterials could require the use of excess amount of antibiotics during transplantation to prevent any infections or microbism. Ag ion has been known for its unique biological properties of antibiosis. We already reported that CaO-SiO₂ gels prepared by a sol-gel method can be used as inorganic fillers for osteoconductive composites because they showed apatite-forming ability in a simulated body fluid. In this study, we synthesized 30CaO70SiO₂(CS) gel powders modified with Ag ions ranging from 0 to 100 ppm to provide antibacterial property. Using the Ag-modified CS powders, composites were prepared by combining with polyetheretherketone (PEEK), which was used as a polymer matrix. Apatite formation in a simulated body fluid was observed on all the composites regardless of Ag contents. The composites consisting of CS modified with Ag ions suppressed multiplication of E.coli. Consequently, CS/PEEK modified with Ag ions not only retained its ability of forming HAp but also gained an antibacterial property.

Key words: Sol-Gel, Ag-30CaO · 70SiO₂ gels, Composite, Polyetheretherketone, Antibacterial property.

Introduction

Ceramic biomaterials have been used to recover the lost functions of the injured bones inside the body by directly fusing with the living bones. Since the development of Bioglass[®], some types of bioactive ceramics including glass and glass-ceramics have been developed for biomedical applications [1, 2]. The bioactive ceramics bond to the living bones, i.e., show osteoconduction, through a bone-like apatite layer formed on surfaces of the composites after implantation in bony defects [3]. It was reported that glasses of CaO-SiO₂ system showed formation of the bone-like apatite layer on surface of the composites in the body environment [4]. Therefore, the CaO-SiO₂ system is well known as essential composition to form hydroxyapatite on its surface in body environment [5]. However, the glasses of CaO-SiO₂ system prepared by a melt and quenching method have limited number of possible composition of Ca/Si to form hydroxyapatite [5]. Besides glass preparation by a melt and quenching method is occurred at high temperature over 1000 °C. To overcome the problem, we suggested sol-gel derived CaO-SiO₂ system

[6], which has capability of manufacturing variety of compositions of Ca/Si.

We reported that the spherical particles of 30CaO · 70SiO₂(CS) gel can be synthesized using a sol-gel method [7], which has two merits such as a capability to synthesize constant and even high-purity material, and a capability to easily manipulate the gel with desired shapes from a porous structure to a spherical particle. The CS spherical particles formed hydroxyapatite on their surfaces after soaking in a simulated body fluid (SBF) that has similar concentration of inorganic ions to human blood plasma according to the study by Kokubo and his colleagues [8, 9]. In addition, the composite prepared from the CS particles and polyetheretherketone (PEEK) showed hydroxyapatite-forming ability in a SBF [10]. In addition, the composites showed similar mechanical property with human cortical bones.

Often, applications of biomaterials could require the use of excess amount of antibiotics during transplantation to prevent infection or microbism. Ag ion has been recognized as its unique biological properties of antibiosis [11]. The effectiveness of Ag ion as an antibacterial agent is based on the ability to irreversibly damage the enzyme systems in the cell membranes of bacteria. There is a need to manufacture a new biomaterial with Ag ion to directly prevent any infection after implantation. In this study, CS gel with 10-100 ppm Ag ions (Ag-CS) was synthesized

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using our previous sol-gel method [12]. The gels were incorporated into PEEK to make osteoconductive composites. The composites were soaked into a SBF to observe apatite formation. Furthermore, we studied multiplication of *E. coli* on the Ag-CS/PEEK composites.

Materials and Methods

Synthesis of Ag-CS gel

Tetraethoxysilane (TEOS, Si(C₂H₅O)₄, Nacalai tesque Inc., Japan), calcium nitrate tetrahydrate (CNT, Ca(NO₃)₃ · 4H₂O, Nacalai tesque Inc., Japan), silver nitrate (AgNO₃, Kanto chemical, Japan) and polyethylene glycol (PEG, Aldrich, USA) with molecular weight of 10,000 were used as starting components, and concentrated nitric acid (62% HNO₃, Wako, Japan) was used as a catalyst for hydrolysis of TEOS.

CS powders were prepared with a starting composition of 30CaO · 70SiO₂ in molar ratio, as described in the previous study [7]. The Ag-CS gels modified with 0-100 ppm Ag ion concentrations were synthesized by the following procedure. PEG, CNT, and AGN were dissolved in ultra-pure water, and then the nitric acid was added. TEOS was added to the aqueous solution under vigorous stirring. After stirring for 20 min, the resulting solution was transferred into a square polystyrene case with its top tightly sealed and then kept at 40 °C for 1 day for gelation and aging. The obtained wet gel was dried at 40 °C for 7 days and then heated at 600 °C for 2 hrs. Hereafter, notation of AgXX-CS (X = 0 ~ 100) was assigned according to Ag contents (ppm). For example, Ag50-CS is the CS particles modified with 50 ppm Ag ions.

Preparation of composites

Ag-CS/PEEK mixtures were produced by physically mixing Ag-CS and PEEK powders in 3/7 volume fraction using a planetary ball mill. In the production of the mixtures, PEEK was the base material and Ag-CS powders were dispersed into the PEEK powder to obtain powder mixtures. The prepared mixtures were compacted by uniaxial pressing at 150 MPa. The composites (Ag-CS/P) were prepared with diameter and thickness of 13 mm and 2 mm, respectively. The specimens were heated at 360-365 °C, polished with #1200 SiC paper, and then washed with ethanol in an ultrasonic cleaner.

Characterization

The prepared gels were characterized by Fourier transform infrared spectroscopy (FT-IR: Nicolet 6700, Thermo Ltd., USA). For FT-IR measurement, the prepared gels were pulverized and were mixed homogeneously with powdered KBr at a sample: KBr mass ratio of 1 : 100. The morphology of the prepared gels was observed under a scanning electron microscope (SEM: 6380LA, JEOL Ltd, Japan). Specimens were coated with gold prior to

SEM observations.

In vitro evaluation of apatite formation

Previous reports revealed that the potential-bonding properties of ceramics could be evaluated by apatite formation on the ceramic surface in a SBF (Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0, SO₄²⁻ 0.5 mmol/dm³(mM)) according to the study by Kokubo and coworkers [8]. A SBF is an acellular solution that has a similar composition of inorganic ions to those of human blood plasma. A SBF was prepared with pH 7.25 at 36.5 °C described in the previous reports [9]. Prepared composites were placed in a container with 30 cm³ SBF to investigate apatite-forming ability. The surfaces of the composites were analyzed with the methods of x-ray diffraction (XRD, X-Pert MPD, Philips, Netherlands) and a scanning electron microscope (SEM) before and after immersion into a SBF for 1 day. The amount of the released Ag ions from the composites was measured by inductively coupled plasma (ICP, Optima-8300, PerkinElmer, USA).

Antibacterial test

Antibacterial property of the composites was evaluated against *Escherichia coli* (*E. coli*, KCTC 1682). The composites in 16 well plates were tested by loading the tested bacteria at a concentration of 10⁵ colony-forming units per 1 ml (CFU/ml). The plates were incubated for 2 and 8 hrs at 36.5 °C. After incubation, the concentration of *E. coli* in the suspension was measured by ultraviolet-visible spectroscopy (UV-Vis, OD600 nm, Lambda 2S, Perkin Elmer). An analysis of variance was used to test the significance of the results, and *p* < 0.05 was considered to be statistically significant.

Results

Fig. 1 shows SEM photographs of the CS particles modified with 100 ppm Ag ions before and after soaking in a SBF for 1 day. From Fig. 1(a), it was confirmed that spherical CS particles with 2-3 μm size were obtained by the sol-gel processing. The composition of Ag ions in the gel did not have any effect on the size or the morphology of the CS particles. After soaking in a SBF, the CS particles formed precipitations on their surfaces. Fig. 2 shows XRD patterns of Ag-CS particles with different concentration of Ag ions before and after soaking in a SBF for 1 day. Amorphous peak = was detected for every Ag-CS particles with different Ag ion content before soaking in a SBF. The peaks assigned to HAp were appeared in every Ag-CS particles after the soaking. The XRD peaks showed the formation of HAp with low crystallinity.

Fig. 3 shows FT-IR spectra of the three different composites before and after soaking in SBF for 0 d to 7 days. Before soaking in SBF, Si-O, Si-OH and Si-O-Si bonds appeared at 1100, 960 and 810 cm⁻¹, respectively,

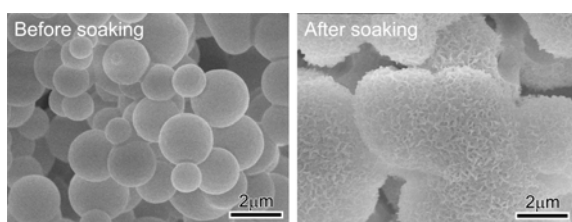


Fig. 1. SEM photographs of the CS particles modified with 100 ppm Ag ions before and after soaking in SBF for 1 day.

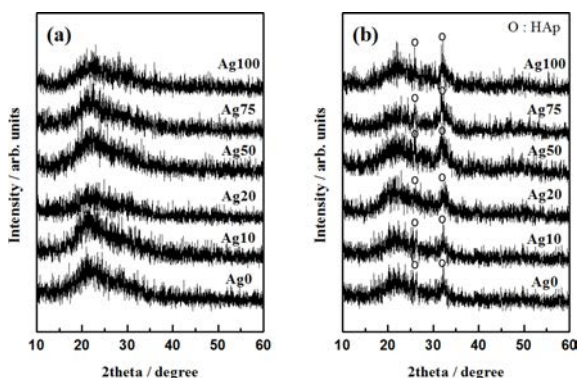


Fig. 2. XRD patterns of Ag-CS particles with (a) before and (b) after soaking in SBF for 1 day.

regardless of Ag ion contents. P-O bonds originated by HAp formation appeared at 1050 cm^{-1} . Fig. 4 shows SEM photographs of the Ag50-CS/P before and after soaking in SBF for 7 days. Before soaking, CS particles on the surface of the composites were clearly observed. New deposition on the surface was observed after soaking in

SBF. The size of precipitation increased and the deposition covered the surface of the composite within 7 days after soaking. The spherical-shaped precipitates had similar morphology with HAp formed on osteoconductive materials after exposure to SBF [10]. Fig. 5 shows XRD patterns of the composites before and after soaking in SBF for 1 day. Some broad peaks assigned to PEEK with semi-crystallinity were detected in every composite both before and after soaking in SBF. The peaks of HAp were appeared after soaking the composites in SBF within 1 day regardless of Ag contents. Any difference in peak intensity and crystalline phase was not observed among the composites after soaking in SBF. Fig. 6 shows SEM photographs of Ag-CS/P with various Ag contents after soaking in SBF 7 days. Spherical precipitation was observed on all the composites regardless of Ag ion contents. As shown in the XRD and the SEM results, Ag ions did not show any effect on apatite formation on the composites.

Fig. 7 shows changes in Ag concentration of SBF after soaking the composites for 7 days. Up to 1 day in SBF, the concentration of Ag ion released from both Ag50-CS/P and Ag100-CS/P increased drastically. The concentration of Ag ion released from Ag50-CS/P increased to approximately 0.15 mg/L , whereas that of Ag100-CS/P increased up to 0.2 mg/L . Then, the Ag50-CS/P began to reach plateau after 1 day in SBF, with slight increase in Ag ion release. In case of Ag100-CS/P, the maximum concentration of Ag ions released from the composites was up to 0.3 mg/L . Comparing to Ag50-CS/P, Ag ions were released from

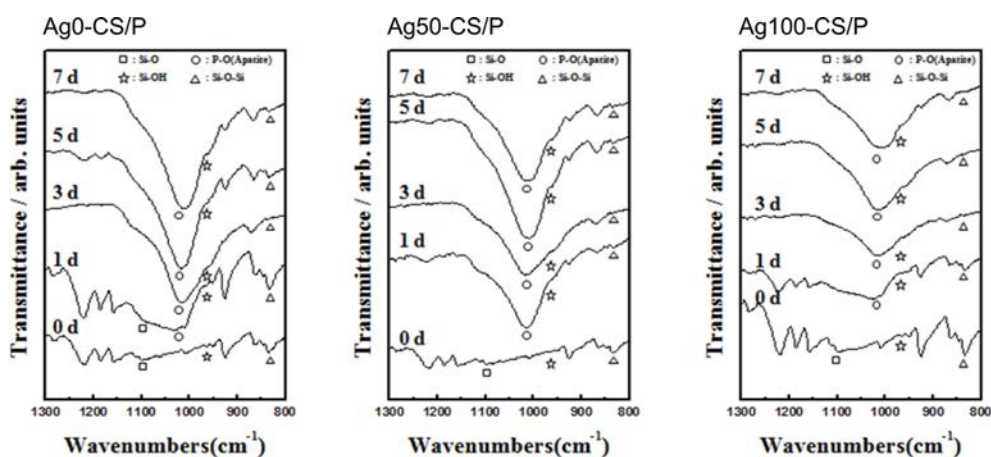


Fig. 3. FT-IR spectra of the composites before and after soaking in SBF for 7 days. 0 day = before soaking in SBF.

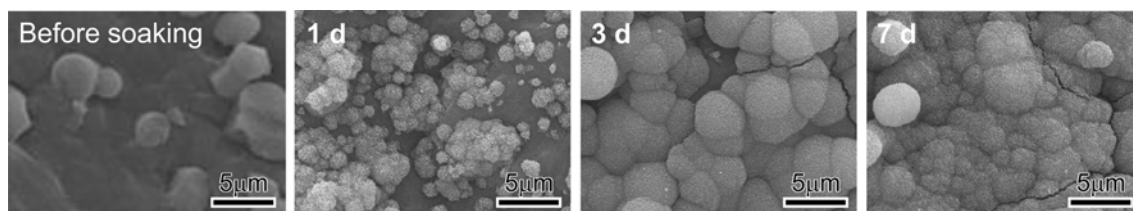


Fig. 4. SEM photographs of the Ag50-CS/P before and after soaking in SBF for various periods.

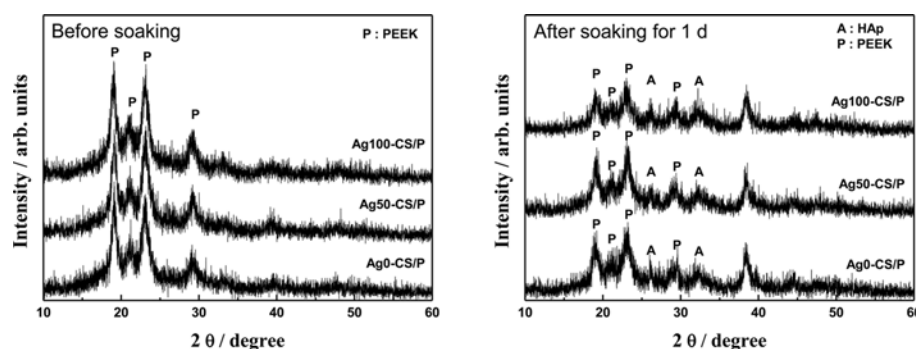


Fig. 5. XRD patterns of the composites before and after soaking in SBF for 1 day.

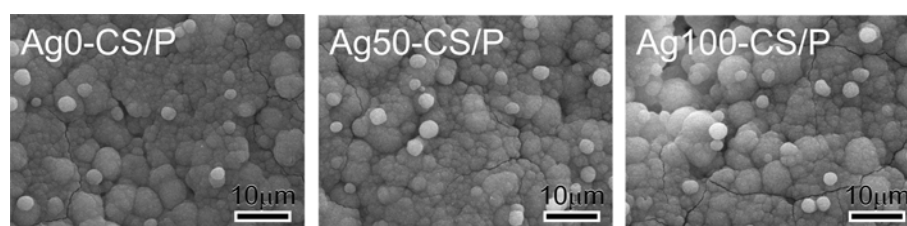


Fig. 6. SEM photographs of Ag-CS/P with various Ag contents after soaking in SBF 7 days.

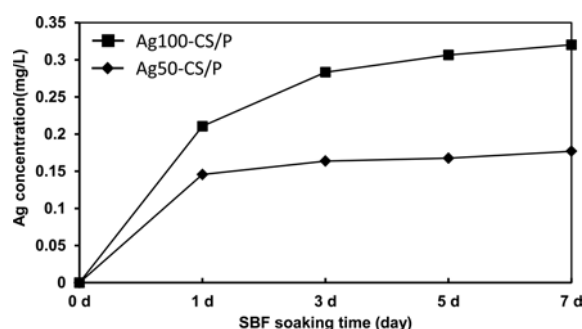


Fig. 7. Changes in Ag concentration of SBF after soaking the composites for 7 days.

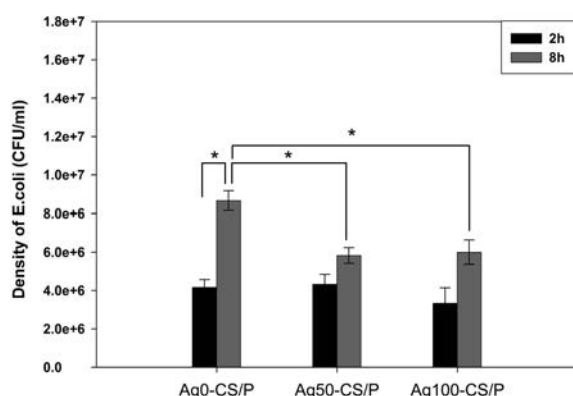


Fig. 8. Changes in density of E.coli on the composites after incubation for 2 and 8 hrs.

Ag100-CS/P was about twice as much as that of Ag50-CS/P. Fig. 8 shows density of E. coli on the composites for 2 hrs and 8 hrs. Density of E. coli on Ag0-CS/P was 4.0 and 8.3×10^6 after incubation for 2 hrs and 8 hrs, respectively. Comparing to Ag free composites, the composites with Ag ions suppressed multiplication of E. coli with higher extent. However, there is no

significant difference in multiplication of E. coli within the composites of different Ag ions contents.

Discussion

In the previous study, we already reported that CS (30CaO-70SiO₂) particles easily formed HAp on their surfaces in SBF within 1 day [7], and the CS spherical particles were introduced as inorganic fillers to fabricate osteoconductive composites []. In Fig. 1, the CS particles with Ag ions were manufactured in size 2-3 μm with spherical morphology by using a sol-gel method. The figure suggested that the size and morphology are independent from the concentration of Ag ions in the particles. Compared to melt-quenched glasses, sol-gel derived CaO-SiO₂ system was more effective on HAp formation due to higher specific surface area; the melt-quenched glasses has flat plate-like morphology with lower surface area according to the previous study [13].

Mechanism of HAp formation on CaO-SiO₂ system is well known. After exposure to SBF, calcium ions are released to surrounding fluid, and at the time silanol group (Si-OH) on the surface of CaO-SiO₂ is formed by release of calcium ions. The formed Si-OH groups act as nucleation sites for HAp. Once HAp nuclei are formed, the formed HAp is enlarged in size by consuming calcium and phosphate ions from surrounding fluid and covers the surface. This mechanism is also supported by Fig. 3. In the figure, the intensity of Si-O bond observed at 1100 cm^{-1} decreased over time. At the same time, the intensity of silanol group (Si-OH) bond and P-O bond increased. This indicates the formation of silanol group supports the formation of HAp, which is represented in P-O bond formation.

Previous studies proved that prepared CS/PEEK composites show similar mechanical property as human bones do [10]. The CS/PEEK composites have been expected as promising bone fixing materials. However, the bacterial infections are considered as the main reason which can lead to the complication after implantation or implant material rejection of the human body. To minimize the risk of any infection, the implant materials covered with biologically compatible materials or antibacterial agents are incorporated within the chemical structure of the implant materials.

Several studies describe that metal ions like Ag^+ have been used for a long time in the biomedical field as antibacterial agents [11, 14, 15]. Metal ions elicit toxic effect on bacteria, algae, and fungi, which is also known as an oligodynamic effect. Among the metal ions, Ag ion was chosen because it is the least toxic to living body among all the metal ions [14]. The combination of Ag ions and CS particles may produce an implant material with capability of preventing infections (Fig. 8). Moreover, the Ag-CS particles were incorporated with PEEK to fabricate osteoconductive composites (Fig 3, 5). After all, Ag modification of CS particles was considered as an effective processing to induce antibacterial property to composites. From ICP results, it was confirmed that Ag ions were released from the CS particles in PEEK matrix (Fig. 7). According to the data of E.coli incubation (Fig. 8), Ag ions suppressed multiplication of E. coli effectively. However, there was no significant effect of Ag ion content on multiplication of E.coli for 2 and 8 hrs. Based on Figs. 7 and 8, Ag ion release was two-fold higher in Ag100-CS/P whereas the magnitude of suppressing the E. coli multiplication between Ag50-CS/P and Ag100-CS/P had no significant difference. These results suggest that Ag ion content of 50ppm in the CS/P complex is enough to elicit an antibacterial property in the case of E.coli.

According to the previous study, ceramic and metal biomaterials such as bioglass [16], HAp [17], TiO_2 [18] and titanium [19] were modified by Ag ions to induce antibacterial property. They reported Ag modification did not affect HAp-forming ability on the materials. In this study, Ag effects on HAp-forming ability of the CS particles and the composites were evaluated. All the composites formed HAp on their surface within 1 day regardless of Ag contents. It means that CS particles show similar extent of HAp-forming ability on their surfaces even the particles were modified with Ag ions, compared to the ones without Ag modification. Besides, the formed HAp on the composites is similar morphology of that on the already reported osteoconductive materials (Fig. 1). As a result, Ag ions did not affect not only HAp-forming ability but also morphology of formed HAp after exposure to SBF.

Conclusions

We synthesized CS gels modified with various amounts of Ag ions to induce antibacterial property, and then applied the CS gels to fabricate composites. The bone-like apatite was formed on the CS gels during the immersion in SBF regardless of concentration of Ag ion, which indicates inclusion of Ag ions did not reduce the functionality of the composites as an osteoconductive composite. Using the Ag-modified CS powder, composites were prepared by combining with PEEK as a polymer matrix. Apatite formation on all the composites after in SBF was observed regardless of Ag contents. The composites consisting of CS modified with Ag ions suppressed multiplication of E. coli. Consequently, CS/P modified with Ag ions not only retained its ability of forming HAp but also gained an antibacterial property.

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