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Design of Novel Bioactive Materials through Organic Modification of Calcium Silicate

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Abstract

Bioactive ceramics have attractive feature for bone repair such as direct bone-bonding in the body. However their clinical application is limited to low loaded portions due to their inappropriate mechanical performances such as higher brittleness and lower flexibility than natural bone. The essential condition for artificial materials to show bioactivity is formation of bone-like apatite on their surfaces in body environment. This apatite formation is triggered by silanol (Si-OH) group on the material surfaces and release of Ca^{2+}. These findings bring us an idea that novel bioactive materials with high flexibility can be designed by organic modification of calcium silicate. We synthesized organic-inorganic hybrids from organic polymers including 2-hydroxyethylmethacrylate (HEMA), starch and alginate by modification with alkoxysilane and calcium chloride. The hybrids formed apatite on their surfaces in simulated body fluid (SBF, Kokubo solution). Such a modification was also effective for providing conventional polymethylmethacrylate (PMMA)-based bone cement with bioactivity.

Keywords; Nanocomposites, Apatite, Silicate, Biomedical applications, Bioactivity

Introduction

Bony defects can be sometimes repaired by own bones extracted from other parts of the patient. The problem is that available bony tissue is limited to the only small amounts. Therefore artificial materials which can repair the bony defects are needed. However, artificial materials implanted into the bony defects are generally encapsulated with a fibrous tissue of collagen[i]. This is a normal reaction for protecting our living body from foreign substances. The implanted material is consequently isolated from the surrounding bone and does not bond to the living bone.

In a few decades, several kinds of ceramics have been found to bond directly to living bone after implantation in bony defects. They are called bioactive ceramics, meaning that they elicit and modulate specific biological activity. Sintered hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) [ii], Na_2O-CaO-SiO_2-P_2O_5 glass named Bioglass® [iii,iv], and glass-ceramic A-W that contains apatite and β-wollasotnite crystals in MgO-CaO-SiO_2 glassy matrix [v, vi] are known as bioactive ceramics. Bone-bonding performance of bioactive ceramics are quite attractive, since tight fixation between the bone and the implanted material is achieved. However, there is limitation on clinical application, because of their inappropriate mechanical properties such as high Young's modulus, low toughness, and brittle character. In addition, development of bioactive materials able to be formed into desired shapes during operation is attractive.

Previous reports showed that the essential condition for artificial materials to exhibit the bone-bonding performance, i.e. bioactivity, is formation of biologically active carbonate-containing apatite on their surfaces when implanted in bony defects[vi,vii]. The same type of apatite formation can be observed on bioactive materials even in a simulated body fluid (SBF, Kokubo solution) with ion concentrations nearly equal to those of human blood plasma[viii,ix]. Bioactivity of artificial materials can be therefore evaluated even *in vitro* by examining its apatite-forming ability in SBF. Compositions effective for exhibiting the apatite formation were fundamentally investigated using glasses in the system CaO-SiO_2-P_2O_5. It was reported that the apatite formation is induced not on the glasses in CaO-P_2O_5 system but in CaO-SiO_2 system[x,xi]. Nucleation of the apatite is triggered by a catalytic effect of silanol (Si-OH) group formed on the surface of the glasses and accelerated by the

release of calcium ion (Ca^{2+}) from the glasses into the solution. This finding brings an idea that organic modification of calcium silicate enables development of novel bone substitutes having bioactivity and flexibility analogous to natural bone.

In this study, we focused on the potential to develop bioactive organic-inorganic hybrids with different mechanical and biological performances by chemical modification of calcium silicate with various organic polymers.

Bioactive organic-inorganic hybrids from MPS and HEMA

We attempted synthesis of bioactive organic-inorganic hybrids by incorporation of MPS ($CH_2=C(CH_3)COO(CH_2)_3Si(OCH_3)_3$) and calcium chloride into 2-hydroxyethylmethacrylate (HEMA, $CH_2=C(CH_3)COO(CH_2)_2OH$). HEMA has high hydrophilicity and high biological affinity, and is used for medical applications as contact lens and coating agent on artificial blood vessels[xii].

MPS and HEMA were dissolved in ethanol with the molar ratio of MPS : HEMA = 1 : 9 at a total concentration of 1 mol/dm^3. The solution of 100 cm^3 was heated at 75°C for 3 h with 0.001 mol benzoylperoxide (BPO) as initiator for polymerization of HEMA and MPS. Then the obtained polymer solution was mixed with 20 cm^3 of ethanol solution containing 0.01 mol calcium chloride ($CaCl_2$). Some of the solutions were added with 1 cm^3 of either 1 mol/dm^3 HCl or 1 mol/dm^3 NH_3 aqueous solution as a catalyst of hydrolysis. The resultant solutions were cast in polypropylene containers and dried at room temperature. After gelation, the gels were continued to dry under ambient condition at room temperature until the weight loss of the sample became less than 2% in 24 h. Hybrids without and with addition of HCl and NH_3 were denoted as “NO, “HC” and “NH”, respectively. Tensile mechanical properties of the hybrids were evaluated using a universal testing machine under ambient conditions according to JIS K7113. Nine specimens were subjected to tensile test for NO, and seven specimens for HC and NH.

The bioactivity of the obtained hybrids was evaluated by examining the apatite formation on their surfaces in SBF (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, and SO_4^{2-} 0.5 mmol/dm^3)[ix]. The solution was buffered at pH 7.25 with tris(hydroxymethyl)

aminomethane ($(CH_2OH)_3CNH_2$) and 1 mol/dm^3 hydrochloric acid at 36.5°C. A rectangular specimen of 10 x 10 x 1 mm^3 in size was cut from those hybrids and soaked in 35 cm^3 of SBF at pH 7.25 at 36.5°C for 7 d. Surfaces of the hybrids before and after soaking in SBF were analyzed by thin-film X-ray diffraction (TF-XRD) and scanning electron microscope (SEM) observation.

Figure 1 shows representative stress-strain curves for the MPS-HEMA hybrids. Their tensile strength increased in the order; HC (0.15 ± 0.02 MPa) < NO (0.68 ± 0.04 MPa) < NH (2.10 ± 0.18 MPa). Their Young's modulus also increased in the same order; HC (0.24 ± 0.02 MPa) < NO (2.65 ± 0.19 MPa) < NH (41.1 ± 1.8 MPa). The Young's modulus of the hybrids is quite similar to those of human cancellous bone (50-500 MPa) and articular cartilage (1-10 MPa). Such hybrids are expected to bring a novel material for reconstruction of cancellous bone and articular cartilage.

SEM photographs of hybrids before and after soaking in SBF for 7 d are shown in Fig. 2. After the soaking, spherical particles were formed on the surfaces of hybrids NO and NH, but not hybrid HC. TF-XRD patterns in Fig. 3 gave peaks assigned to poorly crystalline apatite at about 26° and 32°for hybrids NO and NH after the soaking, but not for hybrid HC.

Our results indicate that the hybrids NO and NH are expected to form the apatite even in the body and bond to living bone. In contrast, hybrid HC does not show bioactivity, in spite of existence of silanol groups and release of calcium ions. Release of HCl in hybrid HC would decrease the degree of supersaturation of the surrounding fluid with respect to the apatite, and consequently suppress the apatite formation. There also remains a possibility that silanol groups did not have appropriate structure by addition of HCl, because structural effect of silica gel on ability of apatite formation is also affected by fabrication process of the silica gel[xiii,xiv].

After the polymerization of MPS and HEMA solution, copolymer consisting of MPS and HEMA was produced. The alkoxysilane groups in the copolymer were hydrolyzed by water from atmosphere or catalysts to form silanol groups during aging and drying process[xv,xvi]. The silanol groups then condensed to form siloxane bonds (≡Si-O-Si≡), that make cross-linking among MPS-HEMA copolymer. When HCl was added to the copolymer solution, linear siloxane chains would be mainly formed by polycondensation of silanol groups[xvi]. In contrast, when NH_3 was added

to the copolymer solution, 3-dimentional siloxane networks would be predominantly formed. Therefore, hybrid NH has a harder character caused by high concentration of siloxane network, in comparison with hybrids NO and HC. Lower Young's modulus of hybrid HC might be attributed to lower concentration of siloxane network structure in the hybrids.

Bioactive starch-based hybrids

On the basis of organic modification of calcium silicate, bioactive organic-inorganic hybrids based on another organic polymer can be developed. Starch is a natural organic polymer that is known as a constituent of various kinds of crop. Starch-based materials are focusing much attention as novel bone substitutes[xvii,xviii]. We attempted preparation of bioactive organic-inorganic hybrids from starch by addition of glycidoxypropyltrimethoxysilane (GPS, $CH_2(O)CHCH_2O(CH_2)_3Si(OCH_3)_3$) and calcium chloride.

Potato starch was dissolved in dimethylsulfoxide. Calcium chloride ($CaCl_2$), GPS and ultrapure water were then added to the solution. The prepared sol was subsequently poured into a teflon dish and kept *in vacuo* at room temperature for 3 d to remove bubbles in the solution. It was then dried at 60°C for 21 d. Mass ratio of starch to the total of GPS and starch was ranged from 0.40 to 0.67, while the molar ratio of $CaCl_2$ to GPS was fixed at 0.05. Molar ratio of H_2O to GPS was fixed at 2.0. The specimen with (starch)/(GPS + starch) = x mass% was denoted as "S x". The obtained homogeneous bulk gels were then soaked in SBF at pH 7.40 at 36.5°C for 7 d. Surface structural changes on the hybrids after soaking in SBF were examined by SEM observation and TF-XRD.

Figure 4 shows SEM photographs of the surfaces of the specimens after soaking in SBF for 7 d. Fine particles were observed on the surfaces of S40 and S50, but not S67. The particles were identified with poorly crystalline apatite by TF-XRD.

Bioactive organic-inorganic hybrids can be designed from starch by addition of appropriate amount of GPS and $CaCl_2$. High content of starch inhibits the apatite deposition in SBF. This would be attributed to low content of Si-OH group on the surfaces of the hybrids. Chemical durability of starch can be controlled according to degree of cross-linking. This means that bioactive

organic-inorganic hybrids with different reactivity with body fluid can be designed from starch.

Development of bioresorbable organic-inorganic hybrids

Design of bioresorbable organic-inorganic hybrids are quite important in the field of bone tissue engineering, since the implanted hybrids must be resorbed at an appropriate speed according to bone regeneration. Alginate, an extract of brown seaweed, is a copolymer of β-D-mannuronic acid and α-L-guluronic acid, and contains many carboxyl groups in its structure. Alginate is a well-known polymer as a biodegradable material applicable to artificial skin and drug delivery systems[xix]. We modified alginate gels with silanol groups as well as calcium chloride. The ability of the apatite deposition was examined in SBF.

Aqueous solution of alginate (supplied from Kimica Co.) with 1 mass% was added with ethylenediamine (EDA) and 3-aminopropyltriethoxysilane (APS, $H_2N(CH_2)_3Si(OCH_2CH_3)_3$) using 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl) and N-hydroxysuccinimide (HOSu) as a catalyst. Concentration of each additive was shown in Table 1. Alginate gels modified with EDA, APS, and both of EDA and APS are denoted as AG-E, AG-S and AG-ES, respectively. The obtained gels were freeze-dried and cut into 10 x 10 mm^2 in size. They were soaked in 1 $kmol/m^3$ of $CaCl_2$ solution for 24 h at 36.5°C. The treated specimens were then soaked in 30 cm^3 of SBF at pH7.25 at 36.5°C. Specimens before and after soaking in SBF were observed under SEM equipped with an energy dispersive X-ray microanalyser (EDX). The structures of the specimens were also characterized using TF-XRD.

Modification of alginate with only APS also gave a bulk gel (AG-S), because alginate could be cross-linked by dehydration of silanol groups derived from APS. EDX spectra showed the existence of Si in AG-S and AG-ES. These results indicate that APS was combined to alginate. Figure 5 shows SEM photographs of the surfaces of the specimens after soaking in SBF in 7 d. Fine particles of apatite was formed only on the surface of AG-S in SBF.

Apatite formation was induced on the alginate gels by incorporation of APS and $CaCl_2$. Silanol groups derived from APS would act as heterogeneous nucleation sites of apatite. Moreover,

incorporated calcium ions were released from the gels and they accelerated the apatite nucleation. Modification of calcium silicate with alginate produces organic-inorganic hybrids exhibiting both bioactivity and bioresorbability.

Bioactive bone cement prepared by organic modification of calcium silicate

We applied organic modification of calcium silicate to development of bioactive materials that can be injected into bony defects. Bone cement consisting of poly(methyl methacrylate) (PMMA) powder and methyl methacrylate (MMA) liquid, in which they are mixed and polymerized, is clinically used for the fixation of implants such as artificial hip joint[xx,xxi]. Significant problems of the PMMA bone cement include loosening at the interface between bone and the cement caused by lack of bioactivity. We therefore attempted preparation of bioactive PMMA bone cements by incorporation of MPS and various calcium salts.

We used PMMA powder with molecular weight about 100,000 and average particle size about 14 μm (Sekisui Plastics Co., Ltd., Tokyo, Japan). The PMMA powder was mixed with a calcium salt selected from calcium chloride ($CaCl_2$), calcium acetate ($Ca(CH_3COO)_2$), calcium hydroxide ($Ca(OH)_2$), calcium lactate ($Ca(CH_3CHOHCOO)_2$) and calcium benzoate ($Ca(C_6H_5COO)_2$) at 20 mass% of the powder. BPO was then added to the powder as a polymerization initiator. MMA liquid was mixed with MPS at 20 mass% of the liquid. N,N-dimethyl-*p*-toluidine (NDT) was then added to the liquid as a polymerization accelerator. Composition of the cements was shown in Table 2. The cement denoted as “Reference” has compositions similar to those of the commercially available bone cement (CMW®-1, Depuy), containing neither MPS nor calcium salt. The powder was mixed with the liquid at a powder to liquid ratio of 1 g/0.5 g at 23±2°C. The paste was shaped to a rectangular specimen 10 x 15 x 1 mm^3 in size. At a half of the setting time of the specimens, they were soaked in 35 cm^3 of SBF at pH 7.25 for 7 d to examine apatite-forming ability. Compressive strengths of the cements without and with exposure to SBF were measured with a universal material testing machine.

Figure 6 shows SEM photographs of the surfaces of the cements modified with MPS and various kinds of calcium salts after soaking in SBF for 7 d. Assembles of fine particles were observed

on the cements modified with $CaCl_2$, $Ca(CH_3COO)_2$ and $Ca(OH)_2$ after the soaking. The formed particles were identified with poorly crystalline apatite by TF-XRD as shown in Fig. 7. Figure 8 shows compressive strength of the cements modified with MPS and various calcium salts before and after soaking in SBF for 7 d. The compressive strength of the modified cements decreased after exposure to SBF, except for the cement modified with $Ca(OH)_2$. Among the cements examined in this study, those modified with $Ca(CH_3COO)_2$, $Ca(OH)_2$ or $Ca(CH_3CHOHCOO)_2$ showed the compressive strength near the lower limit required by ISO5833.

Modification of PMMA cement by incorporation of MPS and appropriate kinds of calcium salts makes the cement capable of apatite formation in the body environment. Solubility of the calcium salt in water decreases in the order; $CaCl_2$ > $Ca(CH_3COO)_2$ > $Ca(CH_3CHOHCOO)_2$ > $Ca(C_6H_5COO)_2$ > $Ca(OH)_2$. The cements modified with calcium salts highly soluble in water have tendency to form the apatite in SBF. It is noted that the cement modified with $Ca(OH)_2$ formed the apatite in SBF, in spite that solubility of $Ca(OH)_2$ is the lowest among the calcium salts used in this study. The pH of the surrounding solution remarkably increased after soaking of the cement modified with $Ca(OH)_2$ in SBF. The increase in pH would accelerate the apatite nucleation, since OH^- is a component of the apatite. These indicate that the increase in pH as well as the release of Ca^{2+} governs ability of the apatite formation on the modified cements.

All the cements but that modified with $Ca(OH)_2$ showed decrease in their compressive strength after soaking in SBF. This is attributed to release of Ca^{2+} ions from the cement into the solution. When Ca^{2+} are rapidly released, pores are formed inside the cement, leading to decrease in compressive strength. Appropriate rate on release of Ca^{2+} is desired to keep high mechanical strength of the cement.

Conclusion

Organic modification of calcium silicate that is essential constituents of bioactive ceramics provides a novel design of various bioactive inorganic-organic hybrids. Apatite-forming ability can be provided by the addition of alkoxysilane and calcium salt into organic polymers with different

biological and mechanical performances. This type of chemical modification can also provide PMMA bone cement with apatite-forming ability. Thus obtained hybrids and the cements are expected to bond tightly to living bone when implanted in the body.

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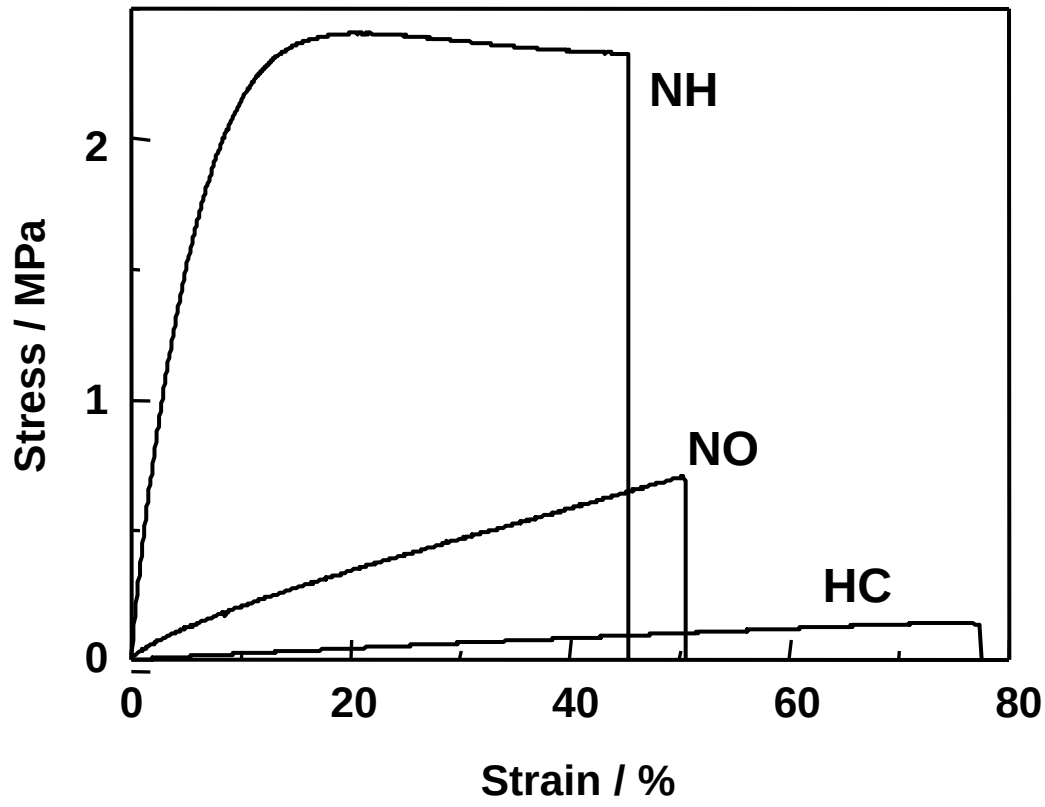

Fig.1 Representative stress-strain curves of the MPS-HEMA hybrids prepared with different catalysts.

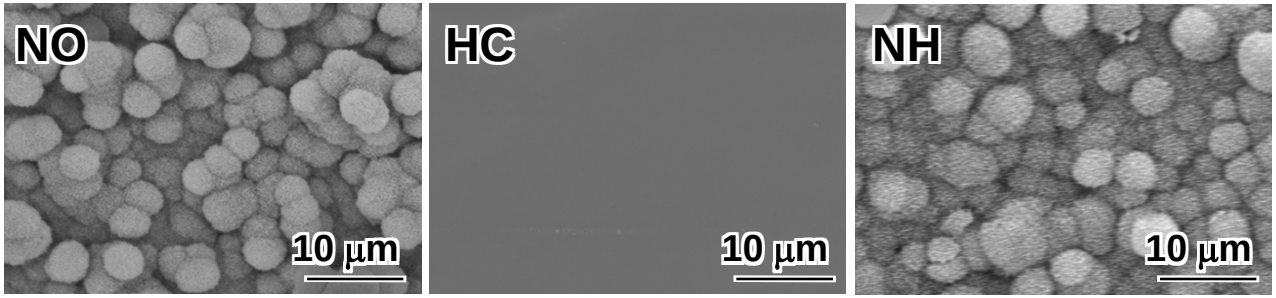

Fig. 2 SEM photographs of the MPS-HEMA hybrids prepared with different catalysts before and after soaking in SBF for 7 d.

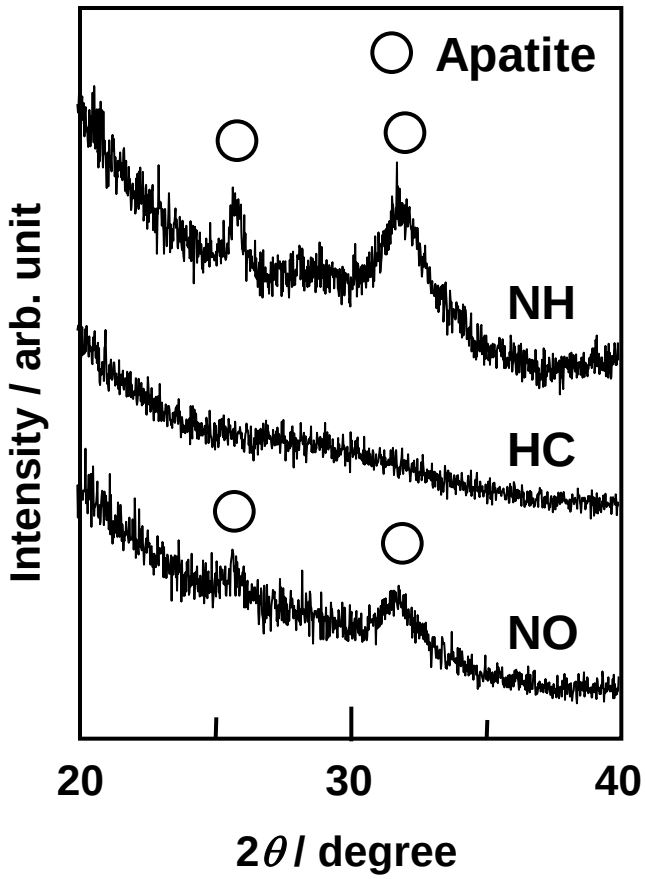

Fig. 3 TF-XRD patterns of the surfaces of the MPS-HEMA hybrids prepared with different catalysts before and after soaking in SBF for 7 d.

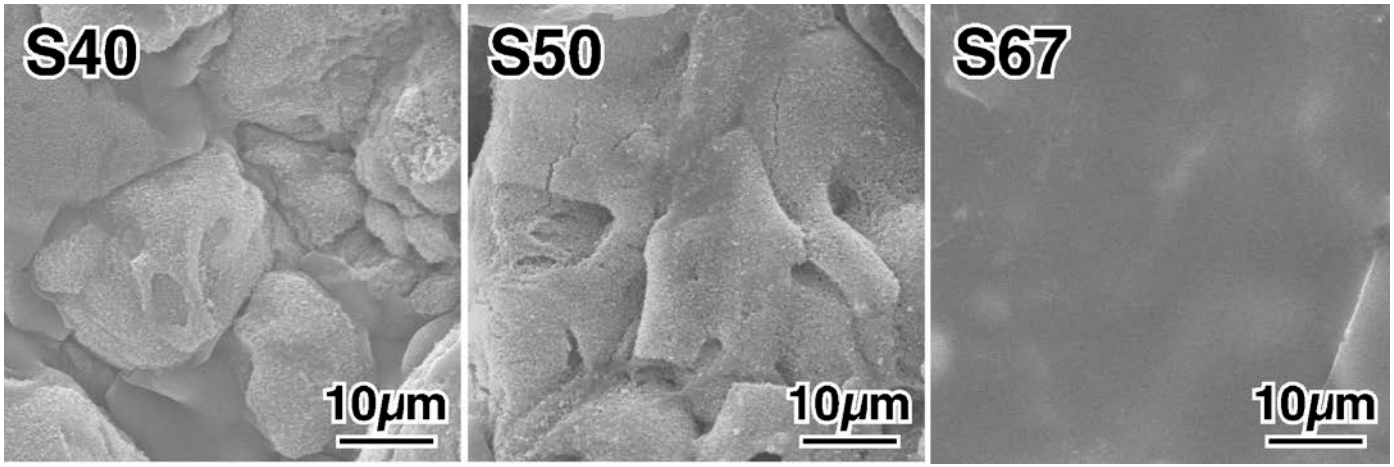

Fig. 4 SEM photographs of the surfaces of GPS-starch hybrids with different starch content after soaking in SBF for 7 d.

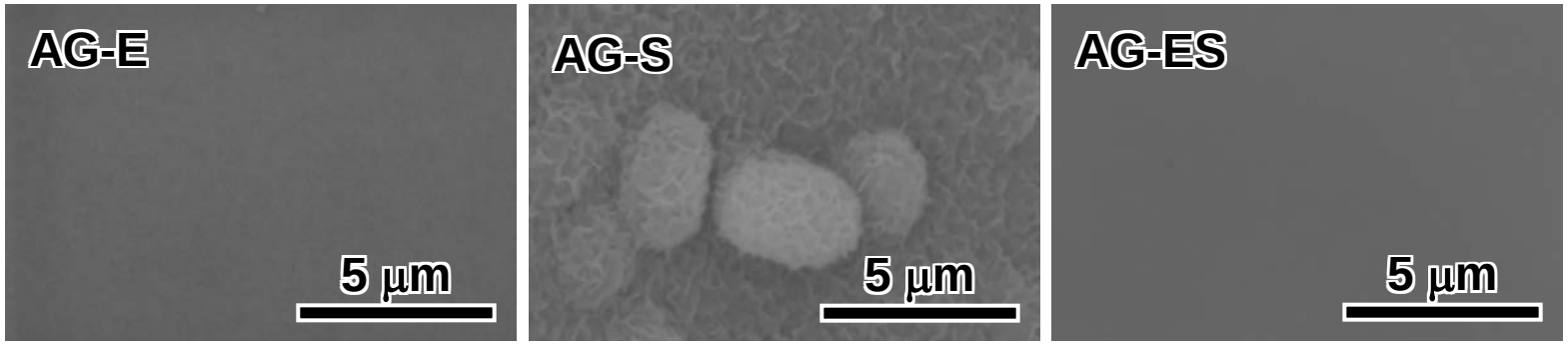

Fig. 5 SEM photographs of the AG-E, AG-S and AG-ES gels surface after soaking in SBF for 7 d.

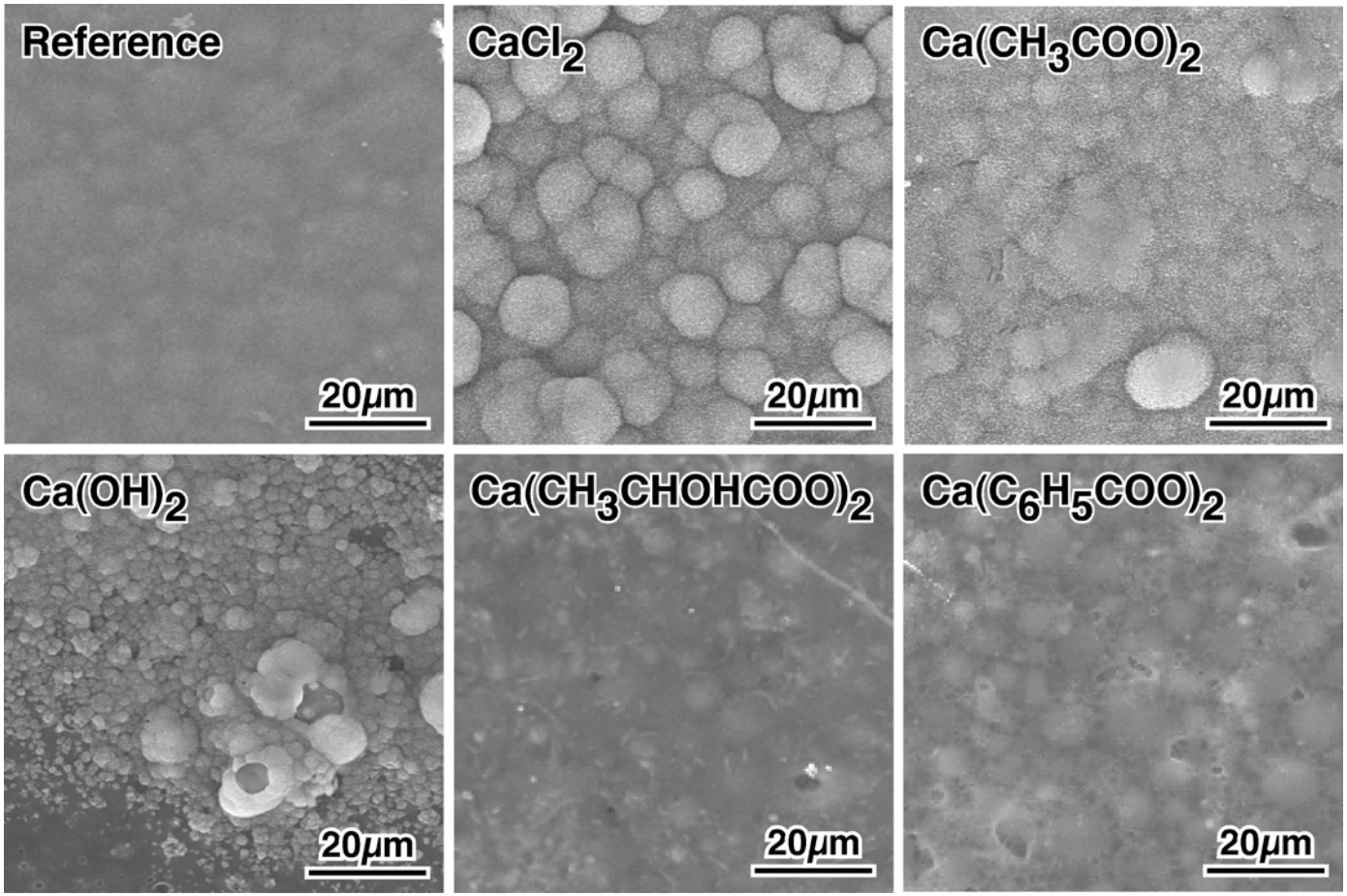

Fig. 6 SEM photographs of the surfaces of the cements modified with MPS and various kinds of calcium salts after soaking in SBF for 7 d.

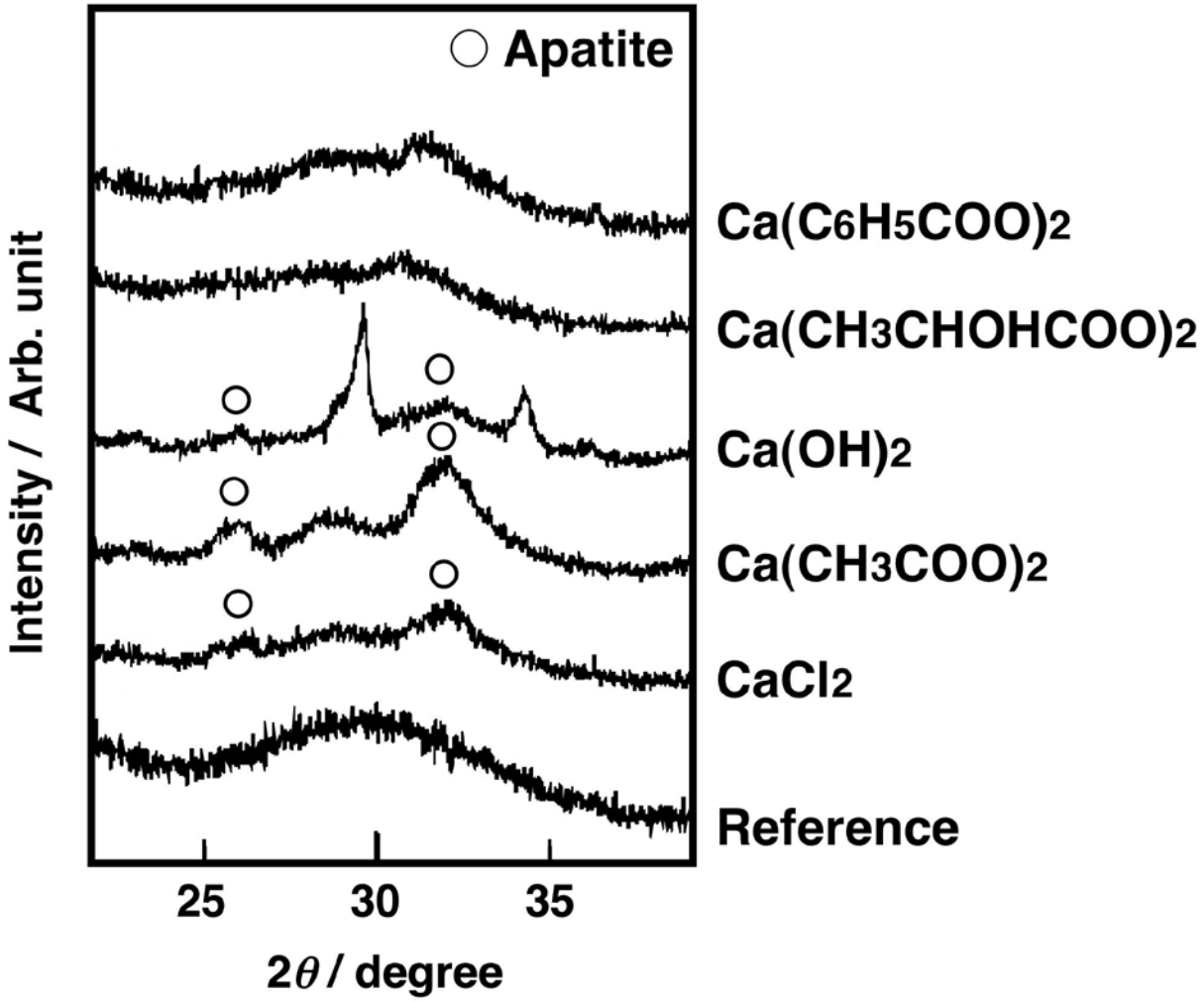

Fig. 7 TF-XRD patterns of the surfaces of the cements modified with MPS and various kinds of calcium salts after soaking in SBF for 7 d.

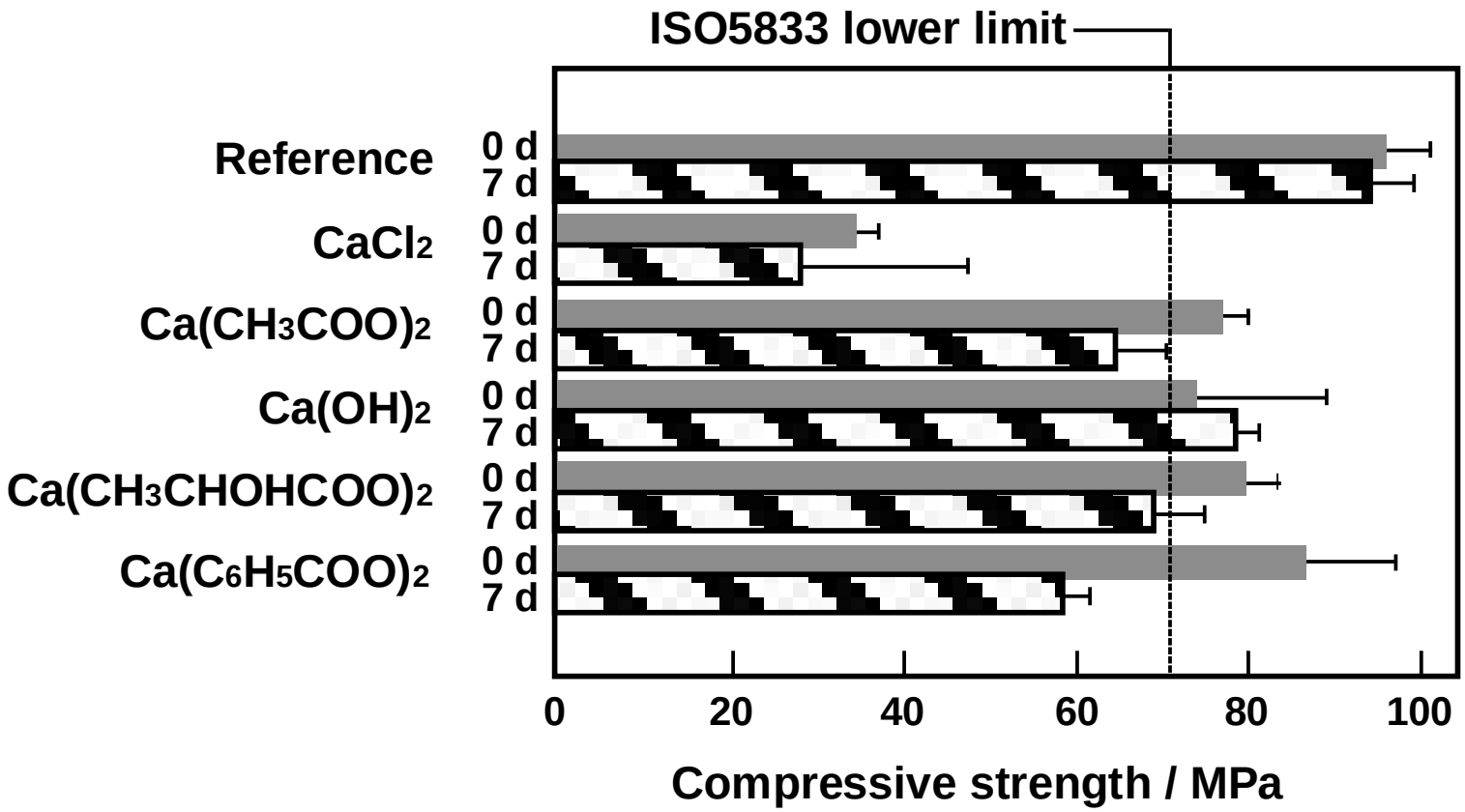

Fig. 8 Compressive strength of the cements modified with MPS and various kinds of calcium salts before (0 d) and after soaking in SBF for 7 d (n=4).