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Relationship between apatite-forming ability and mechanical properties of bioactive PMMA-based bone cement modified with calcium salts and alkoxysilane

Atsushi Sugino · Toshiki Miyazaki · Giichiro Kawachi · Koichi Kikuta · Chikara Ohtsuki

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Abstract Polymethylmethacrylate (PMMA)-based bone cement is used for the fixation of artificial joints in orthopaedics. However, the fixation is liable to loosen in the body, because the cement does not bond to living bone. So-called bioactive ceramics bond directly to living bone through the apatite layer formed on their surfaces in the body. We previously revealed that modification using γ-methacryloxypropyltrimethoxysilane (MPS) and water-soluble calcium salts such as calcium acetate and calcium hydroxide was effective for providing the PMMA-based bone cement with apatite-forming ability in a simulated body fluid (SBF, Kokubo solution) that closely reproduces the body environment. However, the effect of the chemical reaction forming the apatite on the mechanical properties of the cements has not been clarified. The present work aimed to investigate this issue from the viewpoint of the interface structure between the apatite and the cement. The surface of the cement modified with calcium acetate and MPS was fully covered with newly formed apatite after soaking in Kokubo solution within 7 days, while half of the surface area of the cement modified with calcium hydroxide and MPS was covered with the apatite. The bending strength of the modified cements decreased after soaking in Kokubo solution. Porous structure was observed in the region about 50–100 μm in depth from the top surface because of release of the Ca^{2+} ions by both modified cements after soaking in Kokubo solution. The decrease in bending strength of the modified cements could be attributed to the formation of the pores. In addition, the pores on the top surfaces of the cements were filled with the newly formed apatite. The apatite formation would be effective not only for bioactivity but also for decreasing the reduction of mechanical strength.

A. Sugino · G. Kawachi · K. Kikuta · C. Ohtsuki
Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

A. Sugino (✉)
Nakashima Medical Division, Nakashima Propeller Co., Ltd, 688-1, Joto-Kitagata, Okayama 700-8691, Japan
e-mail: a.sugino@nakashima.co.jp

T. Miyazaki
Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu, Fukuoka 808-0196, Japan

1 Introduction

Polymethylmethacrylate (PMMA)-based bone cement has been widely used for the fixation of artificial joints with bone tissues in orthopaedic fields [1, 2]. However, conventional PMMA-based bone cement has several limitations. One of the major limitations is the low biological affinity of PMMA-based bone cement. The cement itself is a typical bioinert material and therefore does not show direct bone bonding, i.e., bioactivity. Thus, the cements may induce aseptic loosening of an implanted prosthesis, because the cement is surrounded by a fibrous tissue capsule after long-term implantation. The loosening allows micromotion of the interface between the cement and the bone to cause failure of prostheses. Therefore, development of bioactive PMMA-based bone cement is highly desirable.

It has been previously reported that bioactive ceramics such as hydroxyapatite, glass-ceramics A-W and titanium dioxide can be added to the powder of the PMMA-based bone cement to provide bioactivity [3–5]. Addition of more than 50 mass% of the ceramics to the cements is required

because most of the ceramic particles are covered with PMMA matrix. This, however, may cause several problems for the workability and Young's modulus of the cements.

An essential requirement for artificial materials bonded directly to bone is the formation of a bone-like apatite layer on their surfaces in the body [6, 7]. The apatite formation is appropriately assessed by using simulated body fluid (SBF, Kokubo solution) with inorganic ion concentrations nearly equal to those of human blood plasma [8]. Ohtsuki et al. closely investigated the mechanism of the apatite formation on $CaO–SiO_2–P_2O_5$ glasses and showed that the apatite formation is successfully induced on the surface of the glass not in a $CaO–P_2O_5$-based system but in a $CaO–SiO_2$-based system [9]. In the $CaO–SiO_2$-based system, Si–OH groups formed on glass surfaces through reaction with the surrounding fluid trigger heterogeneous apatite nucleation, whereas Ca^{2+} ions released from the glass accelerate it by increasing the degree of supersaturation with respect to the apatite. On the basis of these findings, it is expected that chemical modification of the PMMA bone cement with Si–OH groups and Ca^{2+} at a molecular level would decrease the required amount of bioactive constituents. We previously reported that modification of PMMA bone cement with 20 mass% of water-soluble calcium salts that release Ca^{2+} ions, and γ-methacryloxypropyltrimethoxysilane (MPS) that forms Si–OH groups, provides the cement with apatite-forming ability in Kokubo solution [10–13]. Cement of this design type is shown schematically in Fig. 1. The PMMA-based bone cement modified with calcium acetate and MPS exhibited higher bonding strength to the bone than conventional PMMA-based bone cement when they were implanted in beagle femurs [14].

This study aimed to reveal the effect of the apatite formation on the mechanical properties of the modified cements under physiological conditions. We evaluated the four-point bending strength and apatite-forming ability of the cements modified with calcium acetate or calcium hydroxide as well as MPS in vitro using Kokubo solution. The interface structure between the apatite and the cement was carefully observed in order to discuss changes in mechanical properties of the cements in a physiological environment.

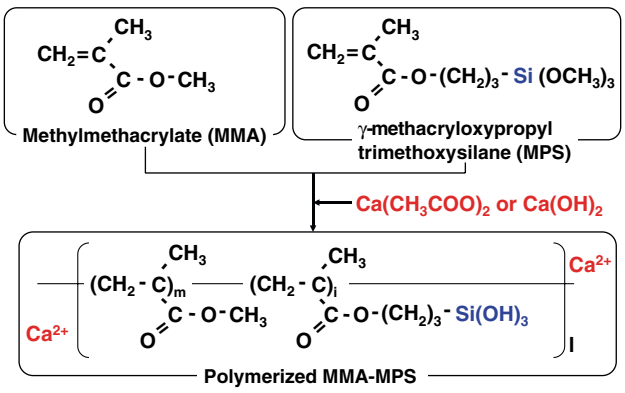

Fig. 1 Design of the PMMA-based bone cement by modification with alkoxysilane and calcium salts

2 Materials and methods

2.1 Preparation of the cement

Calcium acetate monohydrate ($Ca(CH_3COO)_2 \cdot H_2O$, Wako Pure Chemicals Industries, Ltd., Osaka, Japan) and calcium hydroxide ($Ca(OH)_2$, Nacalai Tesque Inc., Kyoto, Japan) were pulverized to a size of <44 μm. In order to remove water in the powder, the former was calcined at 220 °C for 2 h and the latter at 120 °C for 12 h [13]. The PMMA powder with an average molecular weight of 100,000 and 14 μm average particle size was purchased from Sekisui Plastics Co., Ltd., (Tokyo, Japan). The calcium salts were mixed with the PMMA powder. Benzoyl peroxide (Wako Pure Chemicals Industries Ltd., Osaka, Japan) as a radical initiator for polymerization was then added to the mixed powders.

Methylmethacrylate monomer (MMA, Wako Pure Chemicals Industries Ltd., Osaka, Japan) was mixed with MPS (Chisso Corp., Tokyo, Japan) and *N*,*N*-dimethyl-*p*-toluidine (NDT, Kanto Chemical Co. Inc., Tokyo, Japan) as an accelerator of the polymerization. The composition of the powder and the liquid are shown in Table 1. The solely PMMA-based bone cement was also prepared according to the composition of commercially available PMMA-based bone cement. The prepared powder and liquid were mixed by hand with a powder-to-liquid ratio of 2:1 at ambient temperature.

2.2 Evaluation of the apatite-forming ability

The mixed pastes were injected into polypropylene moulds that shaped them into $10 \times 15 \times 1$ mm^3 rectangular specimens. The specimens were then immersed in 35 cm^3 of Kokubo solution before they set and cured. Kokubo solution has ion concentrations of 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, pH 7.25 at 36.5 °C. After soaking the cements for 1, 3 and 7 days, these specimens were taken from the solution, washed gently with ultra pure water and dried at room temperature. They were then characterized by thin-film X-ray diffraction (TF-XRD, M18XHF22-SRA, MAC Science Co., Ltd., Yokohama, Japan) and scanning electron microscope (SEM, S-3500N, Hitachi Co., Ltd., Tokyo, Japan and JSM-5600, JEOL Ltd., Tokyo, Japan)

Table 1 Composition of the examined cements

Sample	Powder/mass ratio			Liquid/Mass ratio		
	PMMA	Calcium salt	BPO	MMA	MPS	NDT
$Ca(CH_3COO)_2$+MPS	0.680	0.194	0.029	0.794	0.198	0.008
$Ca(OH)_2$+MPS	0.680	0.194	0.029	0.794	0.198	0.008
Reference	0.971	0	0.029	0.992	0	0.008

observation. In the TF-XRD, the angle of the incident beam was fixed at 1° against the surface of the specimen, and the measurements were performed using a continuous mode at 2 °/min. For the SEM observation, a thin film of gold was coated on the surfaces of the specimens using a sputtering method.

2.3 Measurement of the bending strength

The bending strength of cements was measured according to ISO5833 [15]. The pastes prepared by hand mixing were moulded to $40 \times 75 \times 3.3$ mm^3. After they completely cured, the cements were cut into $10 \times 75 \times 3.3$ mm^3 rectangular specimens using a micro-cutting machine (MG-400CS, Exakt Apparate GmbH, Norderstedt, Germany). After keeping for 24 h in air atmosphere at 23 ± 2 °C, the surfaces and edges of the specimens were ground with no. 400 silicon carbide abrasive paper. A load was applied to the specimens at a crosshead speed of 5 mm/min using a material testing machine (EHF-F01, Shimadzu Co., Kyoto, Japan) until fracture occurred. The bending strength of the cements, B, was calculated using the equation:

$$B = 3Fa/bh^3 \quad (1)$$

where F is the load at the fracture of specimens, a is the width of upper span, b is the average width and h is the average thickness of the specimens. The thickness and width (b and h) of the specimens were measured before the test. The bending strength results are shown as mean ± standard deviation. Furthermore, the bending strength of the specimens after immersion in Kokubo solution for 7 days at 36.5 °C was also measured. As a comparative material, we used commercial bone cement (Zimmer dough-type radio-opaque cement, Zimmer Inc., IN, USA) as well as a reference sample without MPS or calcium salts.

2.4 Observation of the interface between the apatite and the cement

To observe the cross-section of the cements before and after soaking in Kokubo solution, we used the freeze-fracture technique, reported by Watanabe et al. [16]. The mixed pastes were formed into $20 \times 5 \times 3$ mm^3 rectangular specimens. Specimens of the cured cements were notched at the centre of their underside using a micro-

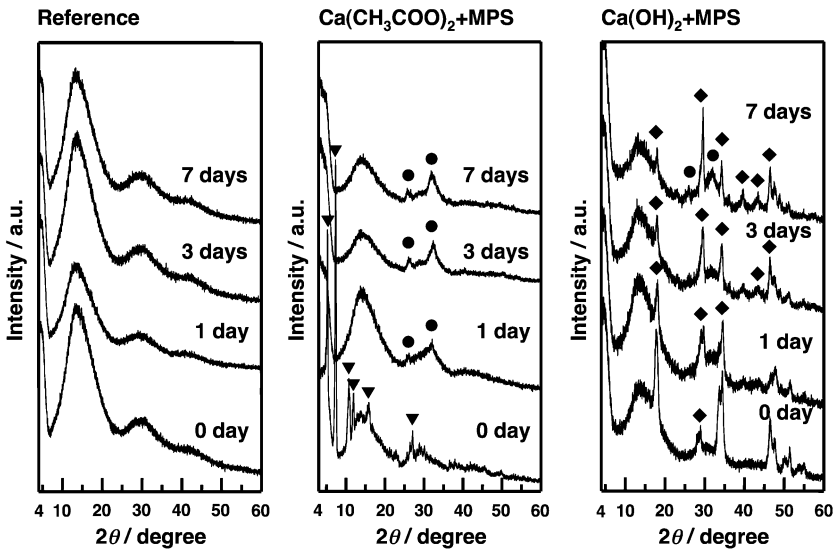

Fig. 2 TF-XRD patterns of the surfaces of the cement samples before and after soaking in Kokubo solution for various periods. (Closed circles: Apatite, Closed triangles: Calcium acetate·0.5H_2O, Closed rhombuses: $Ca(OH)_2$)

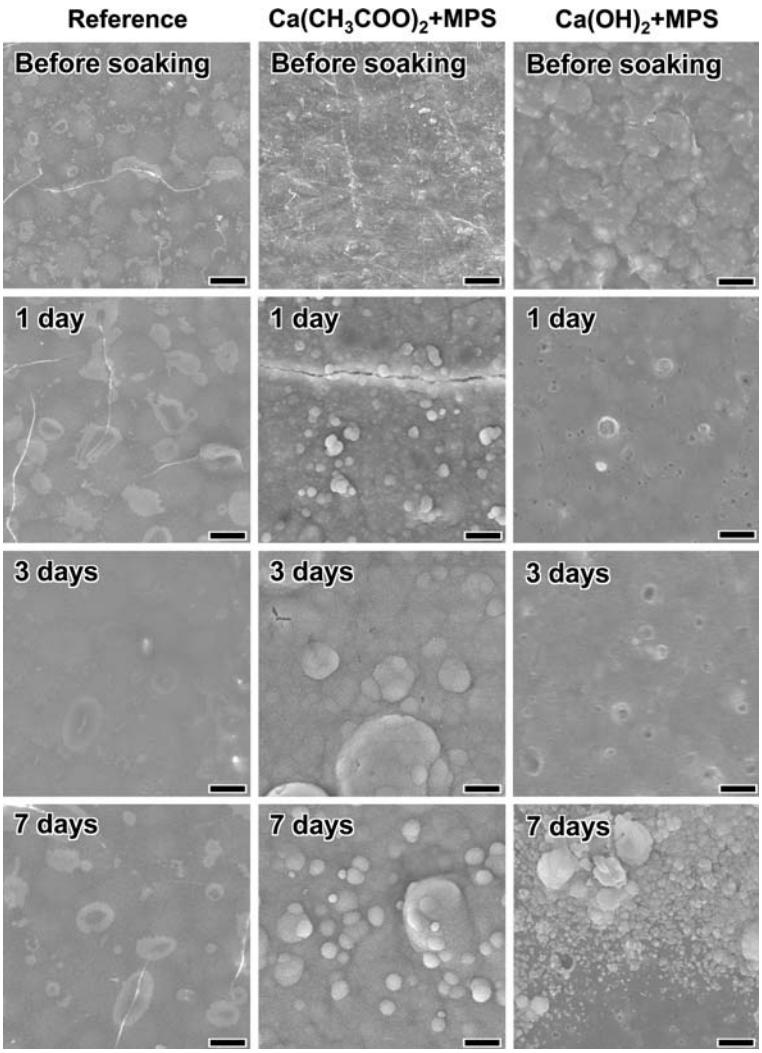

Fig. 3 SEM photographs of the surfaces of the cement samples before and after soaking in Kokubo solution for various periods (Bar: 10 μm)

cutting machine. Then, these samples were soaked in liquid nitrogen for 30 min to fracture them vertically. The cross-sections were characterized using SEM equipped with an energy-dispersive X-ray microanalysis (Super Mini cup, JEOL Ltd., Tokyo, Japan).

3 Results

Figure 2 shows TF-XRD patterns of the examined cements before and after soaking in Kokubo solution for various periods. Broad peaks at 26° and 32° assigned to low-crystalline hydroxyapatite were detected on the surfaces of the cements modified with calcium acetate and MPS within a day after soaking in Kokubo solution. These broad peaks were also observed for the cement modified with calcium hydroxide and MPS within 7 days after soaking. In contrast, any peaks assigned to the hydroxyapatite could not be detected on the reference samples even after soaking in Kokubo solution for 7 days.

Figure 3 shows SEM photographs of the cement samples before and after soaking in Kokubo solution for various periods up to 7 days. An assembly of fine particles covered all of the surface area of the cement modified with calcium acetate and MPS within a day after immersion in

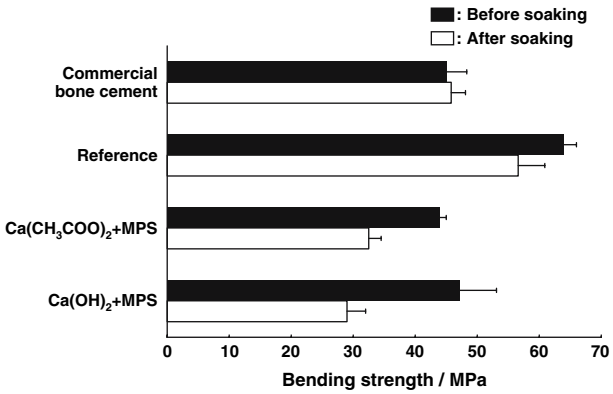

Fig. 4 Four-point bending strength of the cement samples before and after soaking in Kokubo solution for 7 days

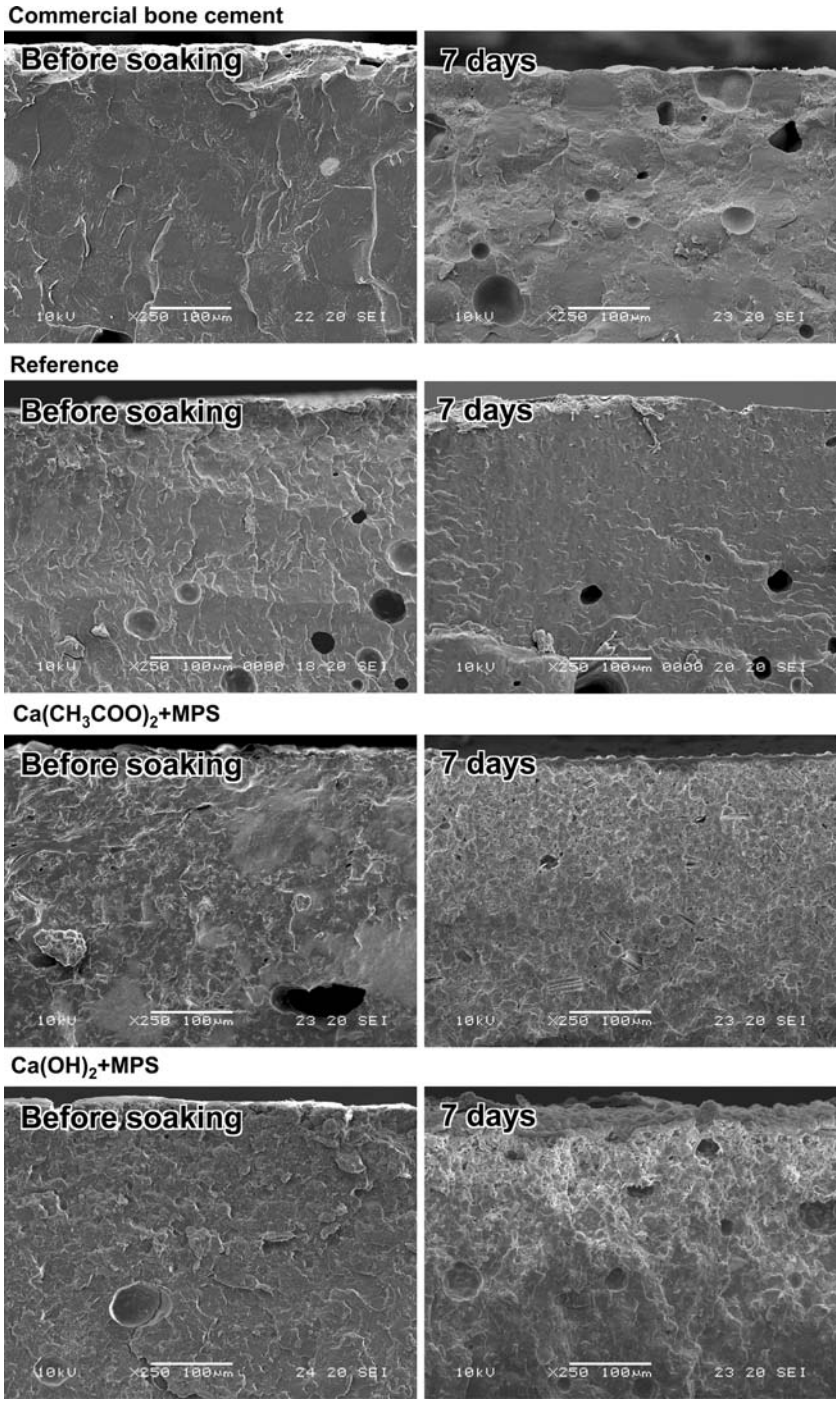

Fig. 5 SEM photographs of the cross-sections of the examined cements before and after soaking in Kokubo solution for 7 days (Bar: 100 μm)

Kokubo solution, while half of the surface area was covered with an assembly of fine particles on the cement modified with calcium hydroxide and MPS even after 7 days. On the other hand, the surface morphology of the reference samples showed no changes even after 7 days. These results demonstrate that the cements modified with calcium acetate or calcium hydroxide as well as MPS forms low-crystalline hydroxyapatite on their surfaces after immersion in Kokubo solution, within a day and 7 days respectively.

Figure 4 shows bending strength of the cement samples before and after soaking in Kokubo solution for 7 days. Before soaking in Kokubo solution both of the modified cements had bending strengths similar to commercial bone cement. The bending strength of the cements almost satisfied the requirement (50 MPa) for clinical applications determined by ISO5833 [15]. The cements modified with calcium acetate or calcium hydroxide and MPS after soaking in Kokubo solution, however, had significantly decreased bending strength

(20%). In contrast, a decrease in bending strength was not observed for either the commercial bone cement or the reference sample.

Figure 5 shows SEM photographs of the cross-sections of the cement samples before and after soaking in Kokubo solution. A few voids because of mixing by hand were observed for all of the cements before soaking. The morphology of the commercial bone cement and reference hardly changed even after soaking in Kokubo solution. In contrast, the cements modified with calcium acetate or calcium hydroxide and MPS showed a lot of pores in the region of 50–100 μm in depth from the top surface after soaking in Kokubo solution for 7 days. SEM photographs and EDX spectra of the cross-sections of the modified cements after soaking in Kokubo solution for 7 days are shown in Fig. 6. The intensity of Ca peaks decreased significantly and then increased slightly with increasing depth for both of the specimens.

4 Discussion

In this study, we examined the apatite-forming ability and mechanical properties of cement modified with MPS and different calcium salts. Apatite formation on the surfaces of the cements modified with calcium acetate or calcium hydroxide and MPS was observed in Kokubo solution within 1 day and 7 days, respectively. We previously reported that the rate of the apatite deposition was strongly affected by the solubility of the calcium salts added to the cements. Namely, the cement modified with calcium acetate with solubility of 34.7 g per 100 g H_2O at 20 °C forms a larger amount of apatite than that modified with calcium hydroxide with a solubility of only 0.17 g per 100 g H_2O at 20 °C [12].

The four-point bending strength of the modified cements after soaking in Kokubo solution increased in the order: $Ca(OH)_2$ and MPS < $Ca(CH_3COO)_2$ and

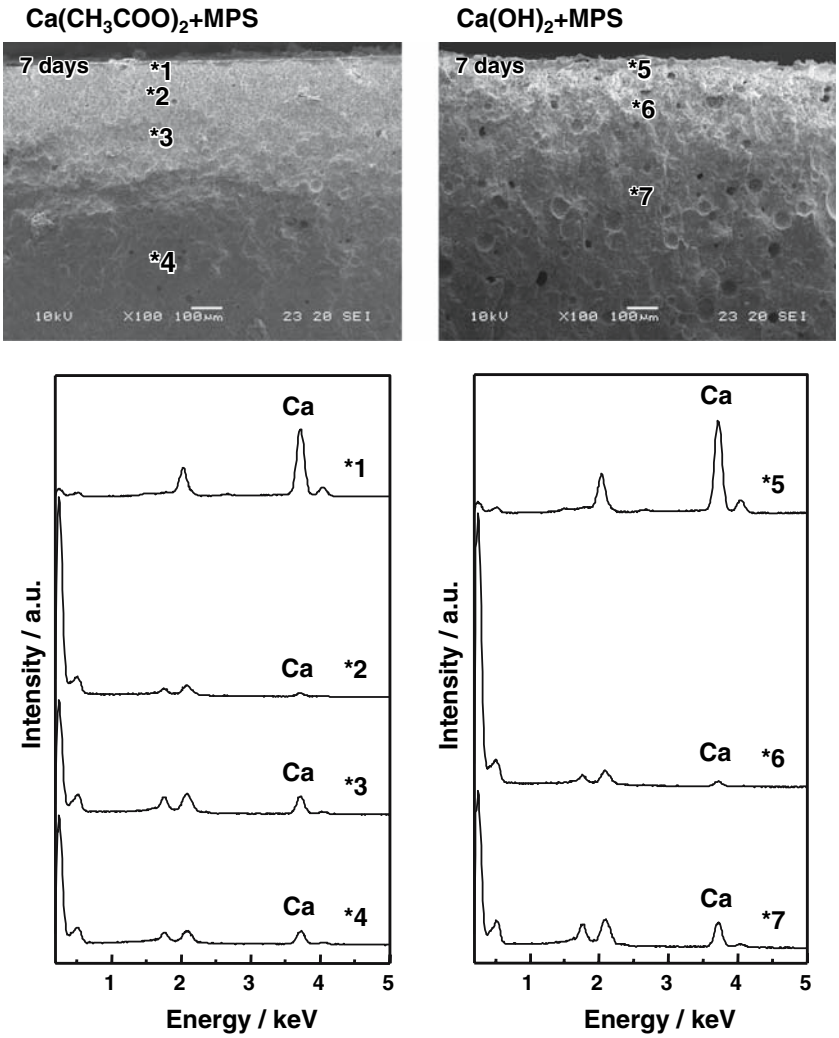

Fig. 6 SEM photographs and EDX spectra of the cross-sections of the modified cements after soaking in Kokubo solution for 7 days (Asterisks: points analyzed, Bar: 100 μm)

MPS < commercial bone cement < Reference (no modification). Both of the modified cements had significantly decreased bending strength after soaking (see Fig. 4). We observed a large amount of the pores in the region of 50–100 μm in depth from the top surface after soaking (See Fig. 5). Moreover, EDX spectra show that intensity of the Ca peak in these regions (point *2 and *6 in Fig. 6) was smaller than that in deeper regions (point *3 and *7 in Fig. 6). The modified cements were designed to release Ca^{2+} ions from the cements into the body fluid to accelerate apatite formation on their surfaces. It is therefore suggested that the decrease in the bending strength of the modified cements in Kokubo solution is attributed to the release of Ca^{2+} ions and subsequent formation of a porous layer with low Ca concentration.

The cement modified with calcium acetate and MPS showed a higher bending strength than that modified with calcium hydroxide and MPS after soaking in Kokubo solution. The former was completely covered with the apatite, while the latter was only partly covered (See Fig. 3). On the top surfaces of the cements, pores were not observed and Ca concentration was highest. Judging from these results, the apatite formed would fill the pores formed by the release of Ca^{2+} ions in Kokubo solution, leading to reinforcement of the cement. Rapid apatite formation in the deeper regions of the cements is quite important, not only for exhibiting bone-bonding ability but also for reducing the decrease in mechanical strength in physiological conditions.

Control of the release of Ca^{2+} into the body fluid should be examined in future work.

5 Conclusion

We examined the relationship between apatite formation and mechanical properties of bioactive PMMA cements obtained by modification using alkoxysilane and water-soluble calcium salts. The cement modified with calcium acetate and MPS formed a larger amount of the apatite on its surface in Kokubo solution than that modified with calcium hydroxide and MPS. The bending strength of both of the modified cements decreased after soaking. This phenomenon was attributed to pore formation in the cements through the release of Ca^{2+} ions. It is also suggested that the apatite deposition in deeper region of the cements would suppress decrease in mechanical strength under physiological conditions by filling the pores.

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