

NOTE

Fabrication of apatite-forming sol–gel hybrids comprising 2-hydroxyethyl methacrylate, 3-methacryloxypropyltrimethoxysilane, and calcium chloride by visible light-induced polymerization

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Silicate-based ceramics have been used to repair bone because they are able to form bone-like apatite upon reaction with body fluids, and to bond directly to bone via the apatite layer. Organic–inorganic hybrids that comprise Ca^{2+} and organic polymers with Si–OH groups, i.e., the basic composition that induces apatite formation, are expected to have mechanical properties similar to those of natural bone. The authors have found that hybrids of 2-hydroxyethyl methacrylate (HEMA) and 3-methacryloxypropyltrimethoxysilane (MPS) with added calcium salts form apatite in simulated body fluid (SBF). However, the hybrid takes more than 1 week to prepare. In the present study, the authors synthesized hybrids with various MPS/(MPS + HEMA) ratios by visible light-induced photopolymerization, and investigated their ability to form apatite in SBF and their mechanical properties. Bulk hybrids were obtained within 3 days at any MPS/(MPS + HEMA) ratio, and they formed apatite in SBF within 3 days. The tensile strength of a hybrid with an MPS/(MPS + HEMA) molar ratio of 0.2 or less was approximately 0.2 MPa, and tended to decrease as the MPS content increased. It was noted that the strength increased up to 2.9 MPa when *N,N*-dimethyl-*p*-toluidine was not added.

Key-words : 3-Methacryloxypropyltrimethoxysilane (MPS), 2-Hydroxyethyl methacrylate (HEMA), Organic–inorganic hybrid, Simulated body fluid (SBF), Apatite, Tensile strength

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Silicate-based glasses and glass ceramics with specific compositions have been used to repair bones because they are capable of bonding directly to bone.^{1),2)} The formation of bone-like apatite by reaction with body fluids is thought to be key to this bone-bonding property.^{1),2)} This apatite formation is induced by surface Si–OH groups and Ca^{2+} release from the material.³⁾ Organic–inorganic hybrids that comprise these inorganic components and organic polymers are flexible and easy to shape. Furthermore, the Young's modulus of such a material can be made close to that of natural bone, preventing stress shielding, in which the surrounding bone becomes brittle owing to the concentration of load on the material. Polymers of 3-methacryloxypropyltrimethoxysilane (MPS),⁴⁾ and hybrids prepared from various polymers such as polydimethylsiloxane,^{5),6)} polycaprolactone,⁷⁾ polyglutamic acid,⁸⁾ and alkoxy silanes made by a sol–gel method reportedly exhibit apatite-forming capability. Moreover, a polymer of 2-hydroxyethyl methacrylate (HEMA) has been used in contact lenses⁹⁾ and artificial heart pumps.¹⁰⁾ The authors of the present

study prepared MPS-HEMA- CaCl_2 hybrids through radical polymerization by heating the components at 75 °C for 3 h, and subsequently allowing the resulting product to age at room temperature.^{11),12)} Apatite formed when the product was brought into contact with simulated body fluid (SBF). However, the preparation of bulk gels takes more than 1 week. The reported MPS/(MPS + HEMA) molar ratio is 0.1 or less. However, the dependence of the properties of the hybrids on their composition remains unclear. Photopolymerization is widely used for dental resins. Hamai et al. prepared HEMA polymers with apatite-forming ability by incorporating phosphate groups after photopolymerization for 1 h and subsequent drying for 1 day.¹³⁾ Based on these findings, in the present study, MPS-HEMA- CaCl_2 organic–inorganic hybrids with various MPS/(MPS + HEMA) ratios were synthesized by photopolymerization. The ability of each hybrid to form apatite in SBF and its tensile strength were subsequently investigated.

The reagents used for SBF preparation were purchased from Nacalai Tesque, Inc., Kyoto, Japan, the MPS was from JNC Co., Tokyo, Japan, and the other reagents were from Fujifilm Wako Pure Chemical Co., Osaka, Japan. MPS, HEMA, benzoyl peroxide (BPO), and *N,N*-dimethyl-

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p-toluidine (NDT) were dissolved in ethanol. Next, (\pm)-camphorquinone (CQ) was added to the mixture, followed by CaCl_2 ethanol solution. Subsequently, the mixture was poured into a plastic mold, polymerized for 2 h while irradiated with blue light (wavelength with maximum intensity of 460 nm), and dried at room temperature for 1–3 days. The molar ratio of MPS/(MPS + HEMA) was varied from 0.1 to 0.9. For example, the sample with an MPS/(MPS + HEMA) ratio of 0.1 is hereafter denoted M1/H9. The molar ratios of BPO, NDT, CQ, and CaCl_2 to the total of MPS and HEMA were 4.6×10^{-4} , 1.6×10^{-3} , 1.33×10^{-3} , and 0.1, respectively. For M2/H8, samples without addition of NDT were also prepared.

The obtained hybrids 10 mm \times 10 mm \times 1 mm in size were soaked in 30 mL of SBF containing 142.0 mM Na^+ , 5.0 mM K^+ , 1.5 mM Mg^{2+} , 2.5 mM Ca^{2+} , 147.8 mM Cl^- , 4.2 mM HCO_3^- , 1.0 mM HPO_4^{2-} , and 0.5 mM SO_4^{2-} at 36.5 $^\circ\text{C}$ for various periods. The pH of the solution was buffered at 7.40 using 50 mM tris(hydroxymethyl)aminomethane and an appropriate amount of HCl. The SBF was prepared in accordance with reported procedures.^{14),15)}

The sample surfaces were characterized by Fourier-transform infrared spectroscopy (FT-IR; FT/IR-6100, JASCO Co., Tokyo, Japan) using an attenuated total reflectance (ATR) method, scanning electron microscopy (SEM; S-3500N, Hitachi Co., Tokyo, Japan), and thin-film X-ray diffraction (TF-XRD; MXP3V, Mac Science Ltd., Yokohama, Japan). The mechanical properties of the samples were examined by tensile tests using a material testing machine (AG-I, Shimadzu Co., Kyoto, Japan) with a cross-head speed of 1 mm/min. The dumbbell-shaped specimens for the tensile test specified in JIS K 6251 (No. 3) were obtained using a punch.

The bulk material was obtained as shown in Fig. 1. Similar bulks were obtained with other compositions. M1/H9 and M2/H8 (No NDT) colored brown and light yellow, respectively. This means that coexistence of BPO, NDT, CQ causes the brownish coloration. Figure 2 shows FT-IR ATR spectra of the hybrids with various MPS contents. Peaks attributable to Si–O–Si, Si–OH, Si–O–CH₃, C–O, C=C, C=O, C–H, and O–H vibrations were observed at 810, 960, 1080, 1200, 1640, 1720, 2840 and 2950, and 3350 cm^{-1} , respectively.^{14),16),17)} The intensity of the Si–O–CH₃ peak increased as the MPS content increased. Figure 3 shows SEM photographs of the hybrids with various MPS contents after immersion in SBF

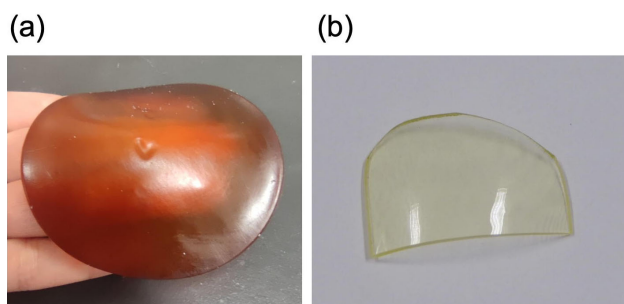


Fig. 1. Appearance of (a) M1/H9 and (b) M2/H8 (No NDT) as prepared.

for 3 days. Flat morphology was observed for all the samples before soaking. Each sample contained aggregates of spherical particles of approximately 2 μm in diameter, and the spherical particles covered the whole surface of the sample. In the TF-XRD pattern shown in Fig. 4, a peak attributable to the 002 diffraction plane was visible at approximately 26 $^\circ$, and envelopes attributable to the 211, 112, and 300 diffraction planes of apatite (JCPDS #09-0432) were visible at approximately 32 $^\circ$. Table 1 shows the tensile strength of the hybrids with various MPS contents. M9/H1 was too brittle to obtain dumbbell-shaped specimens. The tensile strength tended to decrease as the MPS content increased.

Photopolymerization reduced the fabrication time to less than half that of the previous method. In the present study, radical formation occurs both photochemically by CQ and thermally by BPO, whereas in previous studies only the latter occurred. The rate of the radical polymerization is proportional to the square root of the radical concentration.¹⁸⁾ Therefore, the initial polymerization would be enhanced to reduce fabrication time. The obtained hybrid formed apatite within 3 days in SBF (Figs. 3 and 4). FT-IR analysis showed that the peak intensity of Si–OH was smaller than that of Si–O–CH₃ at any MPS content (Fig. 2). Therefore, the Si–OH groups that were capable of inducing apatite formation were mainly formed by hydrolysis by moisture in the air, or by exposure to SBF.

The strength and strain at failure tended to decrease as the amount of MPS increased (Table 1). This is thought to be due to the formation of glassy domains by the polymerization of MPS. In the present study, an MPS with three alkoxy groups around the Si atom was used. However, the polycondensation of the silicate could be reduced and the strength decrease could be suppressed if an alkoxysilane with fewer alkoxy groups is chosen. The tensile strength of each sample added with NDT was lower than the previously reported tensile strength for the MPS-HEMA- CaCl_2 hybrids.¹²⁾ On the other hand, it is noted that the tensile strength of M2/H8 without NDT (2.92 MPa) exceed maximum strength for the previous hybrids (2.15 MPa). In FT-IR, peak intensity of C=C bonds decreased comparing with the sample added with NDT

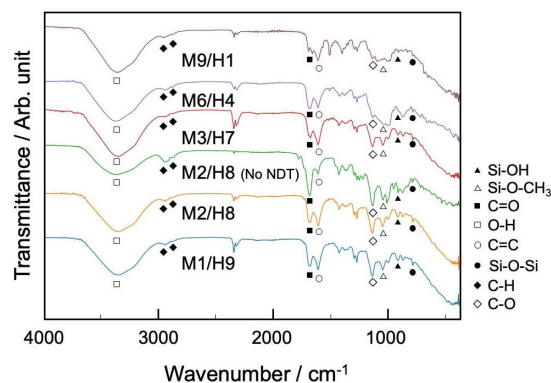
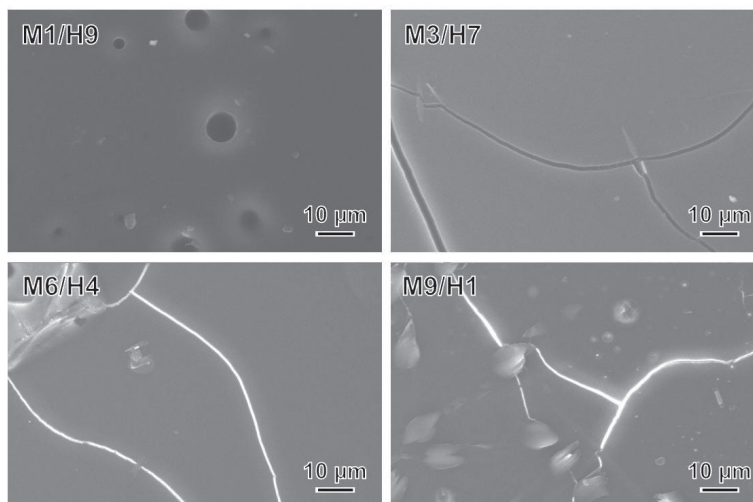


Fig. 2. Fourier-transform infrared spectra obtained by ATR of the hybrids with various MPS contents. (MPS = 3-methacryloxypropyltrimethoxysilane).

Before soaking



After soaking

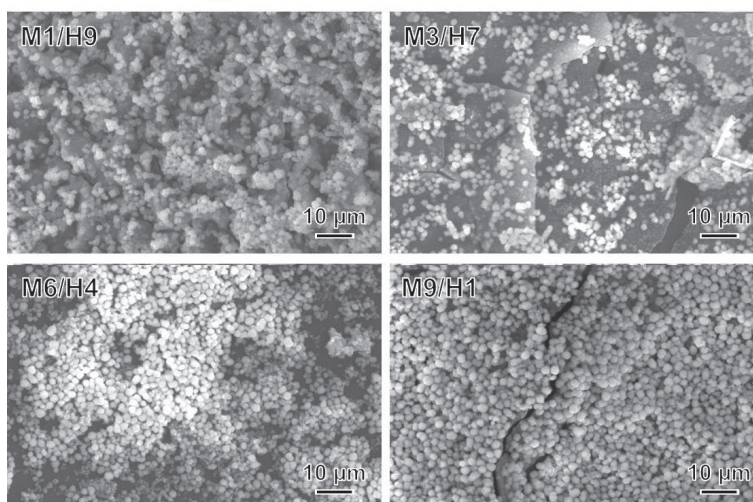


Fig. 3. SEM images of the hybrids with various MPS contents before and after immersion in SBF for 3 days. (SEM = scanning electron microscopy; MPS = 3-methacryloxypropyltrimethoxysilane; SBF = simulated body fluid).

Table 1. Mechanical properties of each sample

Sample	M1/H9	M2/H8	M2/H8 (No NDT)	M3/H7	M4/H6	M5/H5
Number of sample	5	4	4	5	6	3
Strength/MPa	0.160 ± 0.085	0.193 ± 0.092	2.92 ± 0.63*	0.103 ± 0.033	0.079 ± 0.032	0.073 ± 0.037
Strain at failure/%	52.5 ± 12.0	64.9 ± 9.23	44.8 ± 3.11*	27.6 ± 10.4*	23.3 ± 4.88*	11.2 ± 1.58*

*: $p < 0.05$ vs. M2/H8.

(Fig. 2), meaning that radical polymerization is further enhanced. NDT shows a polymerization promoting effect on both CQ and BPO initiators.^{19),20)} However, it is known that the molecular weight of the polymer decreases with increasing radical concentration.²¹⁾ Therefore, it is new finding that the degree of polymerization and the strength of the MPS-HEMA-CaCl₂ hybrids can be improved by appropriate control of the amount of radicals produced.

In the future, it will be necessary to optimize the starting materials and preparation conditions to further increase the

degree of polymerization and improve the tensile strength of the hybrids. In some reports, MPS was hydrolyzed with HCl in advance before photopolymerization.^{22),23)} The degree of polymerization of HEMA is known to vary depending on the intensity of the irradiating light²⁴⁾ and the type of photoinitiator.²⁵⁾ Furthermore, when MPS and CaCl₂ were added to polymethylmethacrylate bone cement to impart apatite-forming capability, the polymerization setting rate decreased as the amount of CaCl₂ increased.²⁶⁾

Judging from the tensile strength, the optimal compo-

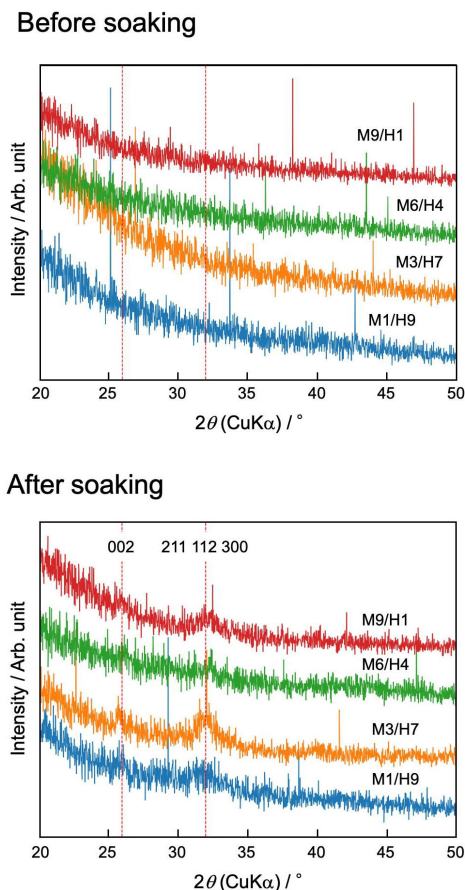


Fig. 4. TF-XRD patterns of the hybrids with various MPS contents before and after immersion in SBF for 3 days. (TF-XRD = thin-film X-ray diffraction; MPS = 3-methacryloxypropyltrimethoxysilane; SBF = simulated body fluid). The sharp peaks, e.g., at 38 and 47° in 2θ for M9/H1 before soaking would be due to electric noise.

sition in this study is considered to be M2/H8, meaning that MPS content can be increased comparing with the previous studies. It is desirable to reduce the amount of calcium in organic-inorganic hybrids to prevent decrease in strength in body environment.²⁷⁾ In the case of MPS-HEMA-CaCl₂ hybrids, even with reduced calcium, the high apatite formation capacity would be maintained due to the formation of large amount of Si-OH group by MPS. Higher strain at fracture (65 %) is one of advantages over the previous report (54 %). The present hybrids are expected to exhibit high ductility even when large amount of inorganic fillers are added to increase the strength.

In conclusion, photopolymerization using visible light was found to remarkably shorten the preparation time of MPS-HEMA-CaCl₂ hybrids. Appropriate control of the polymerization accelerator was found to be an important factor for obtaining the hybrids with high strength.

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