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Immobilization of Semisoft Colloidal Crystals Formed by Polymer-Brush-Afforded Hybrid Particles

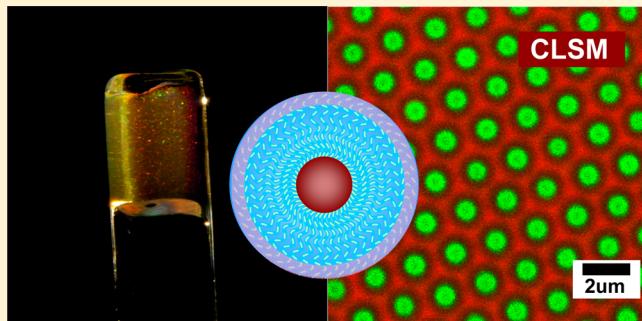
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Supporting Information

ABSTRACT: An immobilization technique for semisoft colloidal crystals, which are ordered arrays of polymer-brush-afforded hybrid particles synthesized by surface-initiated living radical polymerization (SI-LRP), is reported. Silica particles were first grafted with well-defined block copolymers of poly(methyl methacrylate-*co*-hydroxyethyl methacrylate)-*b*-poly(methyl methacrylate), P(MMA-*co*-HEMA)-*b*-PMMA by SI-LRP, which gave a graft density as high as 0.7 chains/nm². The HEMA units reacted with 2-isocyanatoethyl methacrylate to introduce vinyl groups at the outer layer of the polymer-brush shell. The modified hybrid particles formed a colloidal crystal in a solution containing a small amount of free polymers with vinyl groups. The colloidal crystal was photoirradiated in the presence of a photoradical initiator to immobilize it through a cross-linking reaction among the vinyl groups. The structural analyses of the colloidal crystals before and after the photoirradiation were carried out by confocal laser scanning microscopy; the results showed that the periodic structures of the crystals were maintained after immobilization.



INTRODUCTION

Colloidal crystals are three-dimensional periodic arrays of monodisperse colloidal particles, analogous to a conventional crystal where the repeating subunits are atoms or molecules.^{1,2} The driving forces for the formation of colloidal crystals are believed to be repulsive potentials working between colloidal particles. Two types of such potentials are known. One is the hard-sphere potential, which is steric and short-range in nature and induces the crystallization of rigid uncharged particles (hard colloidal crystal).^{3–8} The other is the electrostatic potential, which can be long-range depending on the ionic strength of the system and induces the crystallization of charged particles (soft colloidal crystal).^{9–12} Recently, we identified a colloidal crystal in a suspension of hybrid particles with a shell of well-defined poly(methyl methacrylate) (PMMA) chains densely grafted on spherical silica cores.^{13,14} The driving force of crystallization is the steric or excluded-volume interactions among the polymer chains densely grafted on the particle surface. This system is different from any previously observed colloidal crystals or similar ordered-assemblies formed by polymers of spherical architecture such as block copolymers and star-shaped polymers. This new colloidal crystal was named a semisoft colloidal crystal.^{14–16}

The three-dimensionally periodic structures of colloidal crystals can be used in many interesting applications such as optical filters,^{17–20} sensors,^{21–28} structural color materials,^{29–35} biocatalysts,³⁶ and laser devices.³⁷ However, typical colloidal crystals formed in solution are fragile, and their structures are

easily destroyed by mechanical vibration, evaporation of solvents, and impurities. Therefore, for broadening the applicability of colloidal crystals, it is important that they are immobilized in a way that retains their periodically ordered structure. Asher et al. immobilized colloidal crystals by first adding water-soluble monomers such as acrylamide to a colloidal crystal suspension in an aqueous system and then in situ photopolymerizing them to form a hydrogel entrapping the crystal lattice. They developed an optical sensor with the immobilized colloidal crystals, in which the interparticle distance was changed depending on the hydrogel volume in response to a chemical signal.²¹ Yamanaka et al. immobilized a soft type of colloidal crystals in a polyacrylamide hydrogel and demonstrated that its structural color was tunable by applying mechanical stress. The diffraction-peak wavelength shifted linearly and reversibly according to the compression force over a wide range of visible-light region.³⁶ Takeoka et al. also used a polyacrylamide gel to immobilize colloidal crystals and fabricated a smart membrane that exhibited a structural color controlled by the thickness and volume of the gel membrane.³⁷ Yoshinaga et al. reported a route to immobilize a colloidal crystal of polymer-grafted silica particles by a stepwise procedure comprising gelation by radical polymerization of 1,2-dimethacryloyloxyethane and methyl methacrylate in

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acetonitrile followed by the ring-opening radical polymerization of vinylidene-1,3-dioxolane.³⁸

We herein report on the immobilization technique of semisoft colloidal crystals formed by silica particles densely grafted with a polymer brush. Because the hybrid particles are synthesized by surface-initiated atom transfer radical polymerization (SI-ATRP), the brush layer can be easily modified via polymerization of the functional monomers by making use of the robustness and versatility of living radical polymerization.^{39–43} For crystal immobilization, reactive groups are introduced into a limited part of the polymer-brush layer, and then the colloidal crystal formed by the reactive hybrid particles photoreacts under UV light. The technique is useful for immobilizing semisoft colloidal crystals, and the precise design of the hybrid particles is critical for maintaining the crystal structures before and after immobilization.

EXPERIMENTAL SECTION

Materials. 4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl, >98%) and ethyl bromoisobutyrate (2-(EiB)Br, 98%) were obtained from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Copper(I) chloride (Cu(I)Cl, 99.9%), 4,4'-dinonyl-2,2'-bipyridine (dNbipy, 97%), dibutyltin dilaurate (DBTDL, 90%), hydroquinone monomethyl ether (MEHQ, 99%), anhydrous methyl ethyl ketone (MEK, 99%), Irgacure 907, methanol (MeOH, 99.5%), and tetraethyl orthosilicate (TEOS, 95%) were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan, and were used without further purification. 3-Aminopropyltriethoxysilane (APTS, 98%), 1,2-dichloroethane (99.5%), chloroform (99%), *o*-dichlorobenzene (99%), methyl methacrylate (MMA, 99%), and hydroxyethyl methacrylate (HEMA, extra pure reagent) were obtained from Nacalai Tesque Inc., Osaka, Japan, and MMA was purified by flash chromatography over activated neutral alumina. Silica particle (SiP) (SEAHOSTER KE-E10, 20 wt % suspension of SiP in ethylene glycol) was obtained from Nippon Shokubai Co., Ltd., Osaka, Japan. The mean diameter of the SiP was 130 nm with a relative standard deviation of 10%, as measured by transmission electron microscopy. 2-Isocyanatoethyl methacrylate (MOI) was kindly donated by Showa Denko K.K., Co., Ltd., Tokyo, Japan. Methacryloxyethyl thiocarbamoyl rhodamine B (MTRhoB) was obtained from Polysciences, Inc., Warrington, PA. An ATRP-initiator-holding silane coupling agent, (2-bromo-2-methyl)propionyloxypropyl triethoxysilane (BPE), was synthesized by following a previous report and used to prepare initiator-coated SiPs.⁴⁴ All other reagents were used as received from commercial sources.

Measurements. Gel permeation chromatographic (GPC) analysis was carried out at 40 °C on a Shodex (Showa Denko K.K., Tokyo, Japan) GPC-101 high-speed liquid chromatography system equipped with a guard column (Shodex GPC KF-G), two 30 cm mixed columns (Shodex GPC KF-806L, exclusion limit = 2×10^7), and a differential refractometer (Shodex RI-101). Tetrahydrofuran (THF) was used as the eluent at a flow rate of 0.8 mL/min. PMMA standards were used to calibrate the GPC system. ¹H NMR (300 MHz) measurement was made on a JEOL/AL300 spectrometer (JEOL, Tokyo, Japan). Thermal gravimetric analyses (TGA) were performed on a Shimadzu TGA-50 (Shimadzu, Kyoto, Japan) under a nitrogen atmosphere. Confocal laser scanning microscopic (CLSM) observations were made on an inverted type microscope (LSM 5 PASCAL, Carl Zeiss, Germany) with a 488 nm Ar and a 543 nm HeNe lasers and a $\times 63$ objective (Plan Apochromat, Carl Zeiss).

Preparation of P(MMA-*co*-HEMA)-*b*-PMMA-SiPs. SI-ATRP was employed to obtain SiPs grafted with a well-defined block copolymer of P(MMA-*co*-HEMA)-*b*-PMMA. In the first step: An initiator-coated SiP suspension in MMA containing a prescribed concentration of 2-(EiB)Br and dNbipy was quickly added to a Pyrex glass tube charged with a predetermined amount of Cu(I)Cl (solid). Three freeze-pump-thaw cycles were carried out and the tube was sealed off under vacuum. The polymerization was carried out in a shaking oil bath (TAITEC Corp., Saitama, Japan, Personal H-10) thermostated at 60

°C and, after a prescribed time *t*, quenched to room temperature. An aliquot of the solution was taken out for NMR measurement to estimate monomer conversion and for GPC measurement to determine molecular weight and its distribution. The rest of the reaction mixture was diluted by acetone and centrifuged to collect polymer-grafted SiPs. The cycle of centrifugation and redispersion in organic solvents of acetone/THF/toluene was sequentially carried out, and each cycle was repeated three times to obtain PMMA-grafted SiPs (PMMA-SiPs) perfectly free of the unbound (free) polymer. In the second step, the SI-ATRP block copolymerization was carried out, in the similar way as the first step, in a monomer mixture of MMA and HEMA (mole ratio: [MMA]/[HEMA] = 95/5) in the presence of the macroinitiator PMMA-SiPs obtained in the first step to yield SiP coated with P(MMA-*co*-HEMA)-*b*-PMMA block copolymer.

In a typical run of the first step, the polymerization was carried out at 60 °C for 12 h with the starting materials of MMA (30 g, 300 mmol), anisole (28.1 g), 2-(EiB)Br (189 mg, 0.97 mmol), Cu(I)Cl (76.5 mg, 0.77 mmol), dNbipy (632 mg, 1.55 mmol), and initiator-coated SiPs (2 g), giving a monomer conversion of 73% and a free polymer with $M_n = 22\,700$ and $M_w/M_n = 1.15$, and a graft polymer with $M_n = 23\,000$ and $M_w/M_n = 1.17$. The polymer-grafted SiPs were purified by three cycles of centrifugation (12 000 rpm for 20 min) and redispersion in acetone (3 × 250 mL), THF (3 × 250 mL), and toluene (3 × 250 mL). The purified polymer-grafted SiPs were treated with HF to cleave the graft polymer from the surface of silica particle by following our previous report,¹³ and the molecular weight of graft polymer was determined by GPC.

In a typical run of the second step, the bulk copolymerization was carried out at 60 °C for 3 h with the starting materials of MMA (21.1 g, 210 mmol), HEMA (1.44 g, 11.1 mmol), 2-(EiB)Br (86.5 mg, 0.44 mmol), Cu(I)Cl (87.7 mg, 0.89 mmol), dNbipy (724 mg, 1.77 mmol), and PMMA-grafted SiPs obtained from the first step as mentioned above (1.8 g), giving a monomer conversion of 28% and a free polymer with $M_n = 14\,900$ and $M_w/M_n = 1.28$. The P(MMA-*co*-HEMA)-*b*-PMMA-SiPs were purified by three cycles of centrifugation (12 000 rpm for 20 min) and redispersion in acetone (3 × 250 mL), THF (3 × 250 mL), and toluene (3 × 250 mL). The characteristics of hybrid particles synthesized in this work are summarized in Table 1.

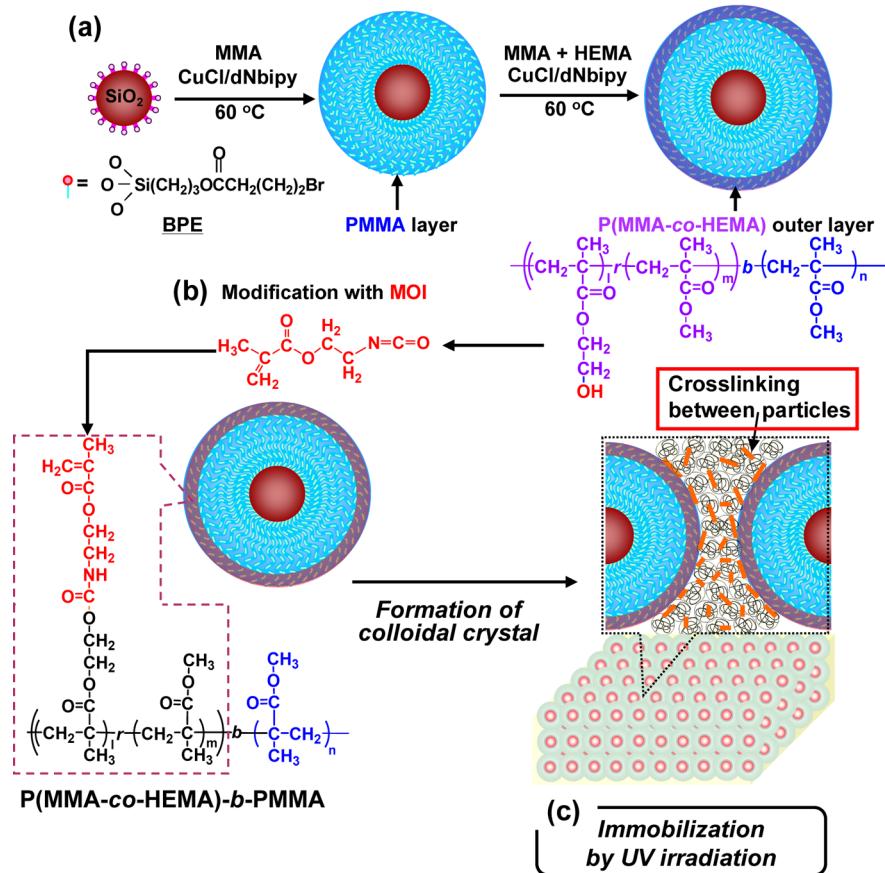
Table 1. Characteristics of Silica Particles Grafted with Block Copolymer Brush

sample code	first block PMMA		second block P(MMA- <i>co</i> -HEMA)			ρ^c (g/mL)	n^d
	M_n^a	M_w/M_n^b	M_n^a	M_w/M_n^b			
P1	23000	1.17	14900	1.28	1.56	1.46	
P2	121000	1.21	14000	1.24	1.28	1.48	
P3	248000	1.13	14900	1.12	1.26	1.49	

^aNumber-average molecular weight of free polymers simultaneously produced in the polymerization. ^bPolydispersity index of free polymers simultaneously produced in the polymerization. ^cCalculated value of the overall (average) bulk density of the hybrid particles. The densities of PMMA and silica particles are known as 1.2 g/mL and 1.9 g/mL, respectively. Using the TGA result and the known densities, the densities of overall hybrid particles were calculated. ^dCalculated value of the overall (average) refractive index of the hybrid particles. On the basis of TGA result, the volume fractions of silica particle and PMMA were calculated with the known refractive indexes of PMMA and silica of 1.49 and 1.44, respectively.

Preparation of Free P(MMA-*co*-HEMA) by ATRP. A free (ungrafted) statistical copolymer of P(MMA-*co*-HEMA) was synthesized by ATRP. The bulk copolymerization was carried out in a shaking oil bath thermostated at 60 °C for 12 h with the starting materials of MMA (18.7 g, 187 mmol), HEMA (1.28 g, 9.83 mmol), 2-(EiB)Br (12.8 mg, 0.066 mmol), Cu(I)Cl (19.5 mg, 0.020 mmol), and dNbipy (161 mg, 0.039 mmol), giving a monomer conversion of 37%

Scheme 1. Schematic Representation for the Synthesis of Hybrid Particles and for the Immobilization of Semisoft Colloidal Crystal: (a) Synthesis of Silica Particles Grafted with a P(MMA-*co*-HEMA)-*b*-PMMA Brush, (b) Synthesis of Vinyl-Group-Carrying Hybrid Particles via Modification with MOI, and (c) Formation and Immobilization of Semisoft Colloidal Crystals



and a free copolymer with $M_n = 112\,000$ and $M_w/M_n = 1.19$. The copolymer was purified by three cycles of reprecipitation using an acetone/MeOH system after the measurement of GPC and NMR. The copolymer was dried in vacuo to remove volatile compounds for 3 h. Yield: 6.9 g.

Introduction of Vinyl Group to Hydroxyl Group of HEMA Unit. MOI (molar ratio of [MOI]/[hydroxyl groups of HEMA units] = 20) was added into the mixture of P(MMA-*co*-HEMA)-*b*-PMMA-SiPs (10 wt % with respect to MEK), DBTDL (molar ratio of [DBTDL]/[MOI] = 2.5×10^{-3}), MEHQ (1 wt % with respect to hybrid particles), and MEK. The mixture was magnetically stirred at 45 °C for 6 h and then for 1 h at room temperature. The reaction was stopped by adding a solvent mixture of MeOH and MEK. The free P(MMA-*co*-HEMA) was also modified in the similar manner.

In a typical run, the reaction was carried out at 45 °C for 6 h with the starting materials of hybrid particles P1 shown in Table 1 (1.74 g, 44 wt % of polymer content, determined by TGA analysis, and 0.013 mmol of hydroxyl groups), MOI (0.41 g, 2.62 mmol), MEK (18 g), MEHQ (7.7 mg, 0.060 mmol), DBTDL (4.14 mg, 0.007 mmol). The reaction was stopped with a mixture of MeOH (0.41 g) and MEK (18 g). The particles were purified by three cycles of centrifugation (12 000 rpm for 20 min) and redispersion in acetone (3×250 mL) and redispersed in toluene to stock.

Formation and Immobilization of Semisoft Colloidal Crystals. Hybrid particles grafted with vinyl-group-carrying polymer brush were dispersed in a mixed solvent in the presence of a prescribed amount of vinyl-group-carrying free polymer. Knowing the weight fraction of polymer in the hybrid particles and literature values of refractive index (n) and density (ρ) of polymer and SiP in the bulk, the overall (average) n and ρ of hybrid particles used here were calculated and summarized in Table 1. The mixed solvents used for the preparation of particle suspensions were adjusted in composition so as

to show the n and ρ values almost equal to those of hybrid particles assuming the additive rule for them.

A Pyrex glass cell connected with a glass tube for sealing was used for the formation and immobilization of colloidal crystal. A solution of photoradical initiator, Irgacure 907, in 1,2-dichloroethane was poured into the cell, and subsequently, the organic solvent was removed in vacuo. A suspension of semisoft colloidal crystal was placed in the cell charged with the dried Irgacure 907 (0.1 wt % with respect to the suspension), and the cell was sealed off. The suspension was mixed by a vortex mixer MS 3 basic (IKA Japan, K. K., Osaka, Japan) and allowed to stand at ambient temperature for the formation of colloidal crystal. The entire operations were carried out in the dark to prevent the onset of photoreaction before the formation of colloidal crystal. The resultant colloidal crystal was exposed to UV irradiation (>300 nm, UV light, MAX-301, Asahi Spectra Co., Ltd., Tokyo, Japan) for 3 h.

In a typical run, P2v particles (1g, see Table 1) were dispersed in a mixture of 1,2-dichloromethane/chlorobenzene with a volume composition of 53/47 (8.834 g, $\rho = 1.18$ g/mL and $n = 1.48$). The free copolymer (0.088 g, 1 wt % with respect to the solvent mixture) was also added into the suspension. A colloidal crystal was formed after standing for 1 h and poured into a Pyrex cell charged with the dried Irgacure 907 (0.001 g). The colloidal crystal formed again in the dark was immobilized by the photoreaction.

Synthesis of Fluorescence-Labeled Silica Particles. The Stöber method was used to synthesize fluorescence-labeled SiPs.^{45,46} APTS (0.14 g, 0.632 mmol) was added, in a glovebox purged with dry argon, into a solution of NBD-Cl (0.12 g, 0.601 mmol) in anhydrous ethanol (15 mL), and the mixture was magnetically stirred for 24 h. A part of the resultant solution (3.28 g) was subsequently mixed with a solution of TEOS (14.7 g) in ethanol (30 mL), and the mixture was

added dropwise at the speed of 7.0 mL/min into a mixture of ethanol (150 mL) and an ammonia solution (28% NH₃ aqueous solution, 36.4 g). The reaction was carried out at room temperature for 3 h with stirring at the speed of 100 rpm. The resultant fluorescence-labeled SiPs were collected by centrifugation (8000 rpm for 10 min) and redispersed in ethanol followed by centrifugation. This cycle was repeated three times to obtain a suspension of fluorescence-labeled SiPs in ethanol. The resultant SiPs were surface-modified with BPE and then with polymer brush by SI-ATRP. The details of the fixation of BPE and the polymerization are described in the Supporting Information.

Synthesis of Fluorescence-Labeled Free Copolymer. The atom transfer radical copolymerization was carried out in bulk at 60 °C for 12 h with the starting materials of MMA (18.7 g, 186 mmol), HEMA (1.28 g, 9.82 mmol), rhodamine-labeled monomer MTRhOB (44.7 mg, 0.007 mmol), 2-(EiB)Br (12.77 mg, 0.0065 mmol), Cu(I)Cl (97.22 mg, 0.98 mmol), and dNbipy (803 mg, 1.96 mmol). The polymerization gave a monomer conversion of 67% and a free fluorescence-labeled polymer with $M_n = 200\,700$ and $M_w/M_n = 1.26$. The free polymer was purified by reprecipitation with acetone/methanol system for three cycles to obtain a fluorescence-labeled copolymer. Yield: 13.3 g. The copolymer was further modified with MOI to introduce vinyl groups, as described above.

RESULTS AND DISCUSSION

Strategy for Immobilization of Colloidal Crystals. Our strategy for the immobilization of colloidal crystals consists of three steps as shown in Scheme 1. First, the hybrid particles are grafted with a polymer brush bearing hydroxyl groups near the free chain-end of the graft polymer, and subsequently, reactive vinyl groups are introduced into the hydroxyl groups. Second, the reactive hybrid particles are employed for the formation of colloidal crystals. Third, the resultant colloidal crystals are photoirradiated by a UV light to form cross-linkages among the vinyl groups. One of the important points of this strategy is the design of the polymer brush. Namely, the reactive sites of vinyl groups should only be placed in a limited part of the polymer brush. This can minimize the volume contraction of the system, possibly caused by the cross-link reaction for the gelation or immobilization of the colloidal suspension. The use of a mild photoreaction condition should also be stressed. These efforts are believed to be necessary to maintain the crystal structure before and after immobilization.

Synthesis of Vinyl-Group-Carrying Hybrid Particles. Hybrid particles grafted with a block copolymer brush of polymer type P(MMA-*co*-HEMA)-*b*-PMMA were synthesized by surface-initiated block copolymerization using MMA and a mixture of MMA and HEMA to prepare the first and second blocks, respectively, as described in the Experimental Section. The characteristics of thus obtained hybrid particles are given in Table 1. Table 1 shows that well-defined, low-polydispersity block polymers comprising PMMA of varying lengths in the first block and P(MMA-*co*-HEMA) of nearly the same length in the second block were successfully grafted on a SiP core with a diameter of 130 nm.

The P(MMA-*co*-HEMA) blocks of the hybrid particles were subsequently modified with MOI to introduce vinyl groups into the hydroxyl groups of the HEMA units. The content of the vinyl groups introduced was too low to be precisely determined even when all the hydroxyl groups were modified. Therefore, we carried out a model experiment using a free (ungrafted) copolymer of P(MMA-*co*-HEMA) containing 5 mol % of HEMA units with $M_n = 112\,000$ and $M_w/M_n = 1.19$.

Figure 1 shows the ¹H NMR spectra of the purified copolymer before and after reaction with MOI. The assignment

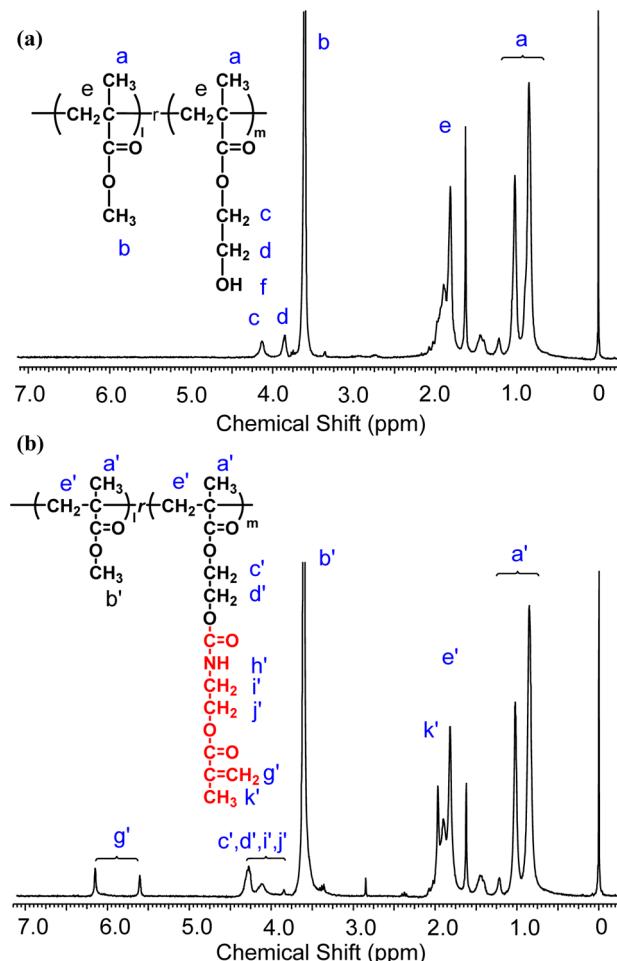


Figure 1. ¹H NMR spectra of free copolymer P(MMA-*co*-HEMA) before (a) and after (b) reaction with MOI. Solvent was CDCl₃.

of the observed peaks is given in the figure. The molar fraction of HEMA units in the copolymer was estimated to be ca. 5 mol % based on the integral ratios of the corresponding peaks in Figure 1a; the value is approximately the same as the molar ratio of HEMA to total monomers in the feed for polymerization, suggesting the formation of statistically random copolymer. In Figure 1b, the two peaks g' at 5.60 and 6.15 ppm are ascribed to the protons of the vinyl groups introduced into the HEMA units. Based on the relative integral of these peaks g' against unchanged a' and b', the molar fraction of the vinyl groups in the copolymer was calculated to ca. 4.6 mol %. This means that almost all the hydroxyl groups of the HEMA units reacted with MOI to obtain the vinyl groups. It can be reasonably assumed that the hydroxyl groups of the graft copolymers have the same reactivity as those of the free polymer and hence that almost all the hydroxyl groups of P(MMA-*co*-HEMA)-*b*-PMMA-SiPs react with MOI, successfully producing hybrid particles with vinyl groups near the free end of the polymer brush.

Immobilization of Semisoft Colloidal Crystals. We prepared a colloidal crystal of vinyl-group-carrying hybrid particles using a *n*- and ρ -matched mixture of solvents containing a photoradical initiator. However, the attempt to immobilize it by UV irradiation did not work satisfactorily. After UV light irradiation, the viscosity of the colloidal crystal suspension increased, and some parts of the suspension formed a gel, which was easily destroyed with gentle shaking of the

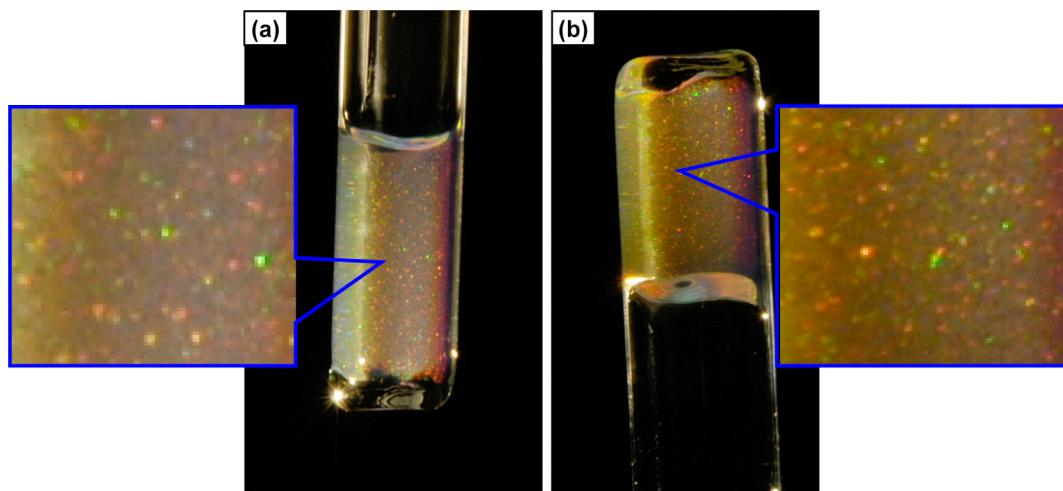


Figure 2. Photographs of a semisoft colloidal crystal in a Pyrex cell illuminated from behind by white light. Images a and b represent the semisoft colloidal crystal before and after UV irradiation, respectively, to immobilize it. Each inset is the close-up view.

system. This indicates that the cross-linking network is more or less formed among the hybrid particles, but that its density was too low to achieve sufficient mechanical strength.

To overcome the difficulty, a free (ungrafted) copolymer with vinyl groups, which has the M_n of 112 000 and the molar fraction of the vinyl group of ca. 5 mol %, was added to promote the cross-linking. To mainly locate the network at the interstitial space among the hexagonally assembled hybrid particles with solvent, the molecular weight of the copolymer was high enough to prevent its deep entry into the polymer brush layer of hybrid particles owing to the size-exclusion effect of the polymer brush as well as low enough to present the macroscopically exclusion (phase separation) from the colloidal crystal.^{39,47}

To examine the effect of free-copolymer addition, a suspension of vinyl-group-carrying hybrid particles, P2v, which are P2 particles (see Table 1) modified with MOI, was prepared by dispersing the particles in a mixture of 1,2-dichloromethane/chlorobenzene with a volume composition of 53/47. One weight percent of the vinyl-group-carrying free copolymer and 0.1 wt % of the photoradical initiator were added beforehand. As shown in Figure 2a, when the suspension of 9.45 vol % particle volume fraction was allowed to stand at ambient temperature, tiny iridescent flecks were observed in the entire volume of the suspension several minutes after the onset of the experiment, indicating the formation of Bragg-reflecting crystallites. The suspension containing the crystallites was subsequently subjected to UV irradiation to initiate a photoradical reaction. Figure 2b shows that the photoirradiated suspension did not flow even in an upside-down glass cell, and the tiny iridescent crystallites were still observed after UV irradiation. This result indicates the gelation of the system, i.e., the immobilization of the colloidal crystal. To tentatively demonstrate the mechanical strength of the crystal, thus immobilized colloidal crystal was taken out of a glass cell and placed in toluene but not destroyed even when the sample was shaken by hand (movie S1 in the Supporting Information).

The immobilization of semisoft colloidal crystals for the suspensions of the hybrid particles of MOI-modified P1 (P1v) and MOI-modified P3 (P3v) was also attempted using a similar technique. A colloidal crystal formed as a whole with a suspension of the P1v at a particle volume fraction of 23.5 vol % in the absence of the free polymers, but it did not form in the

presence of 1 wt % of the free copolymer. This is presumably due to the depletion force and hence the attractive interaction caused between the hybrid particles by the addition of free copolymer. This effect is probably more prominent for the hybrid particles with a shorter polymer chain. When the free polymer concentration was decreased to 0.3 wt %, a colloidal crystal formed at the above-mentioned volume fraction of the particle. Even in such a low concentration of free polymer, the colloidal crystal suspension still became a gel after UV irradiation. In the case of the P3v particles, a colloidal crystal was obtained with a particle volume fraction of 7.13 vol % even in the presence of 1 wt % of the free copolymer. However, the colloidal crystal could not be completely immobilized after UV irradiation. Increasing the free copolymer concentration to 1.8 wt % led to a sufficiently immobilized colloidal crystal. The polymer concentration near the outer surface of the polymer-brush layer was lower because of a relatively long graft chain in comparison with the particle curvature, and more free polymer was needed.

The content of the vinyl groups in the second block of the polymer brush layer is also important for colloidal crystal immobilization. We prepared the hybrid particles of M_n similar to that of the P2v particles but different vinyl-group content of 1 and 20 mol %. Under similar conditions as described above (in the presence of 1 wt % of vinyl-group-carrying copolymers and 0.1 wt % of photoradical initiator), the immobilization of colloidal crystals of these particles was attempted but failed. The hybrid particles with 1 mol % of the vinyl group only gave a mechanically weak gel. In the case of hybrid particles with 20 mol % of the vinyl group, the colloidal suspension was completely immobilized after UV irradiation, but the syneresis that some solvent was excluded on the top of the formed gel was observed because of highly cross-linking resulting in shrinking of gel. These results suggest that the optimization of the vinyl group content is one of the crucial factors for achieving the immobilization of semisoft colloidal crystals.

CLSM Observations. The structural analysis of colloidal crystals was carried out by *in situ* CLSM measurement before and after immobilization. For the high-resolution observation, a suspension of hybrid particles with an appropriate concentration for crystal formation was placed in a glass cell (0.8 cm in diameter and 1.5 cm in height), which had a bottom consisting of a coverslip and a top sealed by gluing a slide glass. Figure

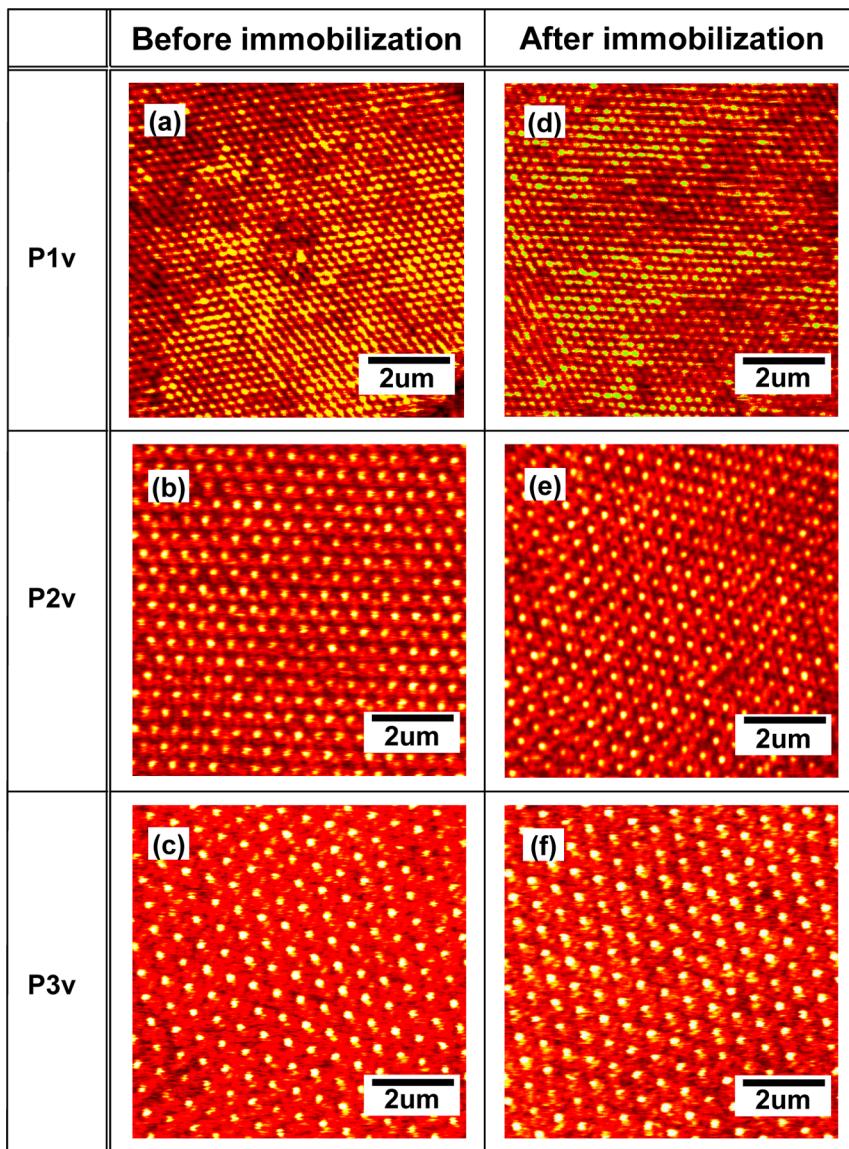


Figure 3. Confocal laser scanning microscopic images of colloidal crystals before and after immobilization. Images a, b, and c show the two-dimensional crystalline planes of colloidal crystals prepared by P1v, P2v, and P3v particles, respectively. Images d, e, and f show the images of the immobilized colloidal crystals prepared by P1v, P2v, and P3v particles, respectively. Observations were performed using an Ar laser of 458 nm wavelength and a 63 \times objective in reflection mode. The distance of the focal plane from the inside of the coverslip was \sim 20 μ m. The diameter of the SiP core is 130 nm. The mean nearest-neighbor center-to-center distances in the images of a, b, and c are 280, 530, and 710 nm, respectively.

3a–c shows the CLSM images of the two-dimensional crystalline planes of the samples prepared by the P1v, P2v, and P3v particles, respectively. The SiP cores of the hybrid particles are clearly visible in all the images as yellow circles forming a two-dimensional hexagonal array, while the polymer brush layers that should be surrounding the SiP cores are hardly visible because of their low reflectivity. An exceptionally high degree in positional order of SiP core and the strong dependence of the interparticle distance on the chain length of the copolymer grafts are particularly noteworthy. Figure 3a has poor resolution because of a smaller interparticle distance, and then an image for the P1v particles was taken and analyzed under higher magnification (Figure 1S in Supporting Information). The mean nearest-neighbor center-to-center distances (D) in the images in Figure 3a–c were measured to be 280, 530, and 710 nm, respectively. In all the samples, the measured D values were larger than the diameter of the

“compact core-shell model”,⁴⁸ which consists of a SiP core and polymer shell of the bulk, and were as large as \sim 50% of the diameter of the “fully stretched core-shell model”,⁴⁸ which consists of a SiP core and polymer shell of the chains radially stretched in all-trans conformation. Those colloidal crystals were irradiated by UV light to immobilize them. Figure 3d–f shows the CLSM images of the immobilized colloidal crystals prepared by MOI-modified P1, P2, and P3 particles, respectively. In all the samples, there was almost no change in the D values of the hexagonally close-packed ordered structures before and after immobilization. These results strongly support the conclusion that the semisoft colloidal crystals were successfully immobilized without disturbing their ordered structures.

To verify the validity of our strategy for the immobilization of colloidal crystals in more details, we attempted to carry out a CLSM measurement in fluorescence mode on an immobilized

colloidal crystal. For this, a fluorescence dye (NBD)-labeled SiPs of a larger diameter of 680 nm was prepared and grafted with a longer polymer brush comprising the first block of $M_n = 301\,300$ and $M_w/M_n = 1.19$ and the second vinyl-group-carrying block of $M_n = 22\,000$, $M_w/M_n = 1.15$, and the mole fraction of vinyl group = 5 mol % (see the Experimental Section and Supporting Information). A rhodamine-labeled, vinyl-group-carrying free copolymer was also synthesized, as described in the Experimental Section, and its M_n and M_w/M_n were 200 700 and 1.26, respectively, and the content of the vinyl group was 5 mol %. A colloidal crystal was prepared using 22 vol % of the NBD-labeled hybrid particles in the presence of 1 wt % of the rhodamine-labeled copolymer in a mixed solvent of 1,2-dichloroethane/chloroform/*o*-dichlorobenzene (57/14/29 volume ratio) and then immobilized in a similar way as the one described above. The sample was observed by CLSM in fluorescence mode by sequential lateral scanning using Ar (488 nm) and He–Ne lasers (543 nm). Figure 4 shows the merged

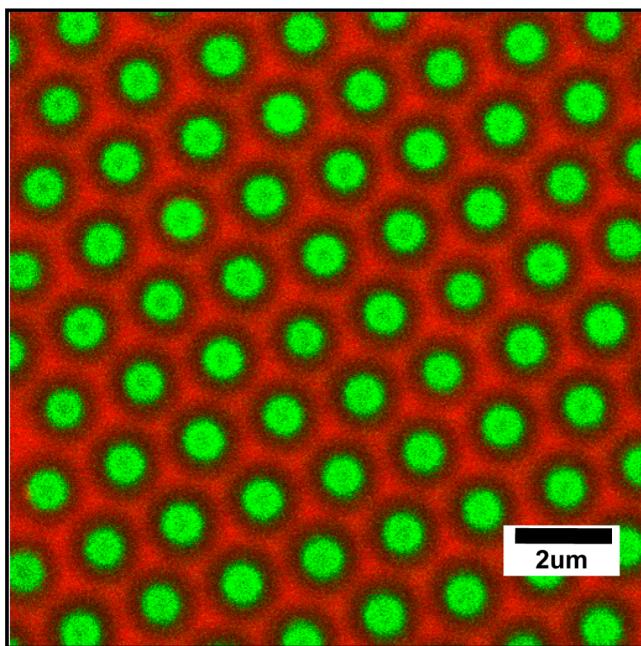


Figure 4. A confocal laser scanning microscopic image of immobilized colloidal crystals formed by NBD-labeled hybrid particles in the presence of rhodamine-labeled free copolymer. The image was constructed by merging two images obtained by sequentially laterally scanning the crystal using an Ar laser (488 nm) and a He–Ne laser (543 nm) with a 63 \times objective in fluorescence mode. Average diameter of the SiP core was 680 nm.

image obtained by the CLSM measurement for the immobilized colloidal crystal using two different lasers. The red and green colors were assigned to the fluorescence emitted by rhodamine molecules of the free copolymer and NBD molecules of the SiP core, respectively. The hexagonally ordered structure of the SiP cores indicated the successful immobilization of the colloidal crystal. It is also noteworthy that the red colors only exist in the interstitial space among the hybrid particles composed of the green SiP core and black (nonlabeled) polymer brush layer. This strongly suggests that the vinyl-group-carrying free copolymer is present in the interstitial space, as expected, among the hybrid particles without completely mixing the polymer brush layer owing to the size-exclusion effect of the polymer brush. It also aids

crystal immobilization by forming cross-linkages among the free copolymer molecules and, to some extent, between the copolymers and the vinyl-group-containing second block of the polymer brush on the SiP core. This phenomenon forms a part of our strategy for immobilizing the semisoft colloidal crystals.

CONCLUSIONS

Semisoft colloidal crystals prepared by polymer-brush-afforded silica particles were successfully immobilized with almost no change in the crystal structure before and after the immobilization. The key to success was the precision design of the polymer brush structure with vinyl-group-carrying blocks of appropriate chain length and the addition of vinyl group-carrying free copolymer with appropriate vinyl group content and molecular weight. The CLSM measurement with a fluorescence-labeled system proved the validity of our strategy for crystal immobilization with the above-mentioned molecular design. The success of immobilization will further broaden the applicability of semisoft colloidal crystals by also considering the versatility and controllability of LRP, which can allow for the construction of a range of colloidal crystals with various functionalities.

ASSOCIATED CONTENT

Supporting Information

Synthesis details for fluorescence-labeled silica particles grafted with block copolymer brush, confocal laser scanning microscopic images of colloidal crystals prepared by P1v particles before and after immobilization, and a movie file showing an immobilized colloidal crystal in solvent. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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